Development of a Film-Forming Oleogel with Increased Substantivity and 3D-Printed Microneedle Array Patches to Improve the Topical Treatment of Psoriasis

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Larissa Carine Pünnel

aus Neuss

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1. Berichterstatter/-in:	Prof. Dr. Dominique Jasmin Lunter
2. Berichterstatter/-in:	Prof. Dr. Rolf Daniels

„Wollen heißt wagen und beharren“

Meiner Familie

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Summary

Psoriasis is a chronic inflammatory disease that affects about 2% of the world's population. This condition is characterized by a red, silver-scaly skin appearance due to the inflammatory processes produced by the rapid turnover of skin cells. The skin barrier is therefore disrupted which leads to increased water loss, painful lesions and itchy skin. Effective treatment requires topical formulations that provide a large number of lipophilic components to treat the disease and hydrate the skin. The two active ingredients that established themselves as the gold standard are the glucocorticoid betamethasone dipropionate (BDP) and the vitamin D derivate calcipotriole (CA) and are available in in-market formulations. Unfortunately, the disadvantage of these formulations is that they can be easily removed from the skin through contact with clothing or other areas of the skin which leads to an insufficient effect of the active ingredients and to an insufficient compliance in long-term therapy. The ability of a formulation to remain on the surface of the skin for a long time is called substantivity. In order to increase patient compliance and drug safety, the aim of this work was to improve the topical therapy of psoriasis by developing a novel film-forming oleogel designed to increase substantivity on the skin.

Several working groups are already involved in the development of topical formulations with increased substantivity. The current products are formulated as emulsions or solutions with a suitable film former and solvent, plasticizers are added if necessary. Since the skin of psoriasis patients is already very dry, these formulations with a high water content are unsuitable for this application.

The OleoCraft™ film-formers consist of polyamides with different average molecular weights. The OleoCraft™ MP-30, with a molecular weight of 30 000 Da, creates a gel network with its polyamide linker when molten up with medium to high polarity oils. One of the key issues in development is the creation of a formulation that both remains well on the skin and from which the active ingredients penetrate the skin in a comparable way to the in-market products. This work has developed an oleogel formulation that incorporates a blend of castor oil, medium-chain triglycerides and OleoCraft™ MP-30 in a concentration of 7%. This formulation forms a cohesive, adhesive film upon application ensuring prolonged adherence to the skin and an increased penetration of BDP into the skin comparable to in-market formulations.

Another part of this work was the development of microneedle array patches (MAP) for the treatment of psoriasis. In general, the penetration of BDP and CA from topical

treatments is hampered due to the thickened skin of the psoriasis. To address these challenges, microneedles are a promising approach. They create microchannels in the skin, which enhance drug absorption and efficacy by increasing the delivery of active pharmaceutical ingredients (API) to the deeper layers of the skin. The use of MAPs may not only improve treatment outcomes, it also offers a minimally invasive and patient-friendly alternative to systemic therapies holding significant potential for better management of psoriasis.

In this work, round microneedle patches (MAPs) with a flexible patch were developed using 3D printing. The microneedles were designed as square pyramids, cones and obelisks with varied lengths of 400 µm, 600 µm, 800 µm and 1000 µm. These MAPs were characterized for force to fracture, penetration depth into the skin and possible skin damage. The obelisk-shaped microneedles proved themselves to be the most robust. Ex vivo experiments proved that applying these MAPs with lengths of 600 µm, 800 µm, and 1000 µm, in combination with the developed film-forming formulation, significantly increased the penetration of BDP out of the formulation into the skin.

Zusammenfassung

Psoriasis ist eine chronisch-entzündliche Hauterkrankung, von der etwa 2 % der Weltbevölkerung betroffen sind. Die Haut ist durch ein rotes, silbrig-schuppiges Aussehen gekennzeichnet, das auf entzündliche Prozesse in der Haut und die Hyperproliferation von Hautzellen zurückzuführen ist. Die Hautbarriere ist daher gestört, was zu einem erhöhten Wasserverlust, schmerzhaften Läsionen und juckender Haut führt. Eine wirksame Behandlung erfordert eine topische Behandlung in Form von Formulierungen, die eine große Menge an lipophilen Komponenten zur Behandlung der Krankheit und zur Hydratisierung der Haut bereitstellen. Die beiden Wirkstoffe, die sich als Goldstandard etabliert haben, sind das Glukokortikoid Betamethasondipropionat (BDP) und das Vitamin-D-Derivat Calcipotriol (CA) Diese Kombination ist in marktüblichen Formulierungen erhältlich. Leider haben diese Formulierungen den Nachteil, dass sie durch Kontakt mit der Kleidung oder anderen Hautpartien leicht von der Haut entfernt werden können, was zu einer unzureichenden Wirkung der Wirkstoffe und zu einer unzureichenden Therapietreue der Patienten bei einer Langzeittherapie führt. Die Fähigkeit einer Formulierung, lange Zeit auf der Hautoberfläche zu verbleiben, wird als Substantivität bezeichnet. Um die Therapietreue der Patienten und die Arzneimittelsicherheit zu verbessern, zielt diese Arbeit darauf ab, die topische Therapie der Psoriasis durch die Entwicklung eines neuartigen filmbildenden Oleogels zu verbessern, das die Substantivität auf der Haut erhöht.

Mehrere Arbeitsgruppen befassen sich mit der Entwicklung von topischen Formulierungen mit erhöhter Substantivität. Bisher wurden filmbildende Formulierungen als Emulsionen oder Lösungen mit einem geeigneten Filmbildner und Lösungsmittel entwickelt, und bei Bedarf Weichmacher zugesetzt. Da die Haut von Psoriasis-Patienten bereits sehr trocken ist, sind diese Formulierungen mit einem hohen Wassergehalt für diese Anwendung ungeeignet.

Die OleoCraft™-Filmbildner bestehen aus Polyamiden mit unterschiedlichen durchschnittlichen Molekulargewichten. Das OleoCraft™ MP-30 mit einem Molekulargewicht von 30 000 Da bildet mit seinem Polyamid-Linker ein Gel-Netzwerk, wenn es mit mittel- bis hochpolaren Ölen aufgeschmolzen wird. Die größte Herausforderung dabei ist, eine Formulierung zu entwickeln, die lange auf der Haut verbleibt und aus der die Wirkstoffe in vergleichbarer Weise und Menge zu den verfügbaren Fertigarzneimitteln in die Haut penetrierten.

Die in dieser Arbeit entwickelte Oleogel-Formulierung enthält eine Mischung aus Rizinusöl, mittelkettigen Triglyceriden und OleoCraft™ MP-30 in einer Konzentration von 7 %, die beim Auftragen einen kohäsiven, haftenden Film bilden, der eine längere Haftung auf der Haut und eine mit marktüblichen Formulierungen vergleichbare Penetration von BDP in die Haut gewährleistet.

Ein weiterer Teil dieser Arbeit war die Entwicklung von Mikronadel-Array-Pflastern (MAP) für die Behandlung von Psoriasis. Im Allgemeinen ist das Eindringen von BDP und CA aus topischen Behandlungen aufgrund der verdickten Haut der Psoriasis eingeschränkt, was ihre therapeutische Wirkung reduziert. Mikronadeln sind ein neuer und vielversprechender Ansatz, um diese Herausforderung zu bewältigen. Sie erzeugen Mikrokanäle in der Haut, was die Abgabe von pharmazeutischen Wirkstoffen an die tieferen Hautschichten erhöht und so die Aufnahme und Wirksamkeit der Arzneistoffe verbessert. Der Einsatz von MAPs verbessert nicht nur die Behandlungsergebnisse, sondern bietet auch eine minimalinvasive und patientenfreundliche Alternative zu systemischen Therapien und birgt ein erhebliches Potenzial für eine bessere Behandlung der Psoriasis.

In dieser Arbeit wurden mittels 3D-Druck runde Mikronadel-Pflaster (MAPs) mit einer flexiblen Basis entwickelt. Die Mikronadeln selbst wurden als quadratische Pyramiden, Kegel und Obelisken mit den Längen von 400 µm, 600 µm, 800 µm und 1000 µm gestaltet. Diese MAPs wurden hinsichtlich ihrer Bruchfestigkeit, der Penetrationstiefe in die Haut und möglicher Hautschädigungen hin untersucht. Dabei erwiesen sich die obeliskförmigen Mikronadeln als die robustesten. Bei der Anwendung von MAPs mit diesem Design in den Längen von 600 µm, 800 µm und 1000 µm in Kombination mit einer filmbildenden Formulierung konnte in ex-vivo-Experimenten eine signifikant höhere Menge an BDP, dass aus der Formulierung in die Haut penetrierte, festgestellt werden.

Aim

In all stages of psoriasis treatment, topical therapy, in the form of ointments and creams, is a central component of the clinical guidelines. In addition to the penetration of active ingredients to reduce inflammatory reactions and inhibit hyperproliferation, existing formulations also offer potential benefits through the vehicle effect where the formulation base itself may positively affect the diseased skin areas. Since the patient's skin is very dry, these formulations contain a high proportion of lipophilic components to support moisture retention and skin barrier repair.

The challenge with the use of these topical treatments is their poor substantivity. Due to low adherence to the skin surface, formulations are easily rubbed off, which leads to a reduced therapeutic efficacy and negatively impacts patient compliance. Additionally, the loss of the formulation can result in contamination of the patient's surroundings.

The main objective of this work was to enhance psoriasis treatment and improve therapeutic outcomes by developing a semi-solid formulation with a high content of lipophilic components that remains on the skin longer, i. e. with enhanced substantivity. The active ingredient combination of 0.64 mg/g BDP 0.05 mg/g CA has established itself as the gold standard for topical treatment. The active ingredients are intended to achieve a comparable skin penetration level as existing marketed formulations.

In addition, the potential use of microneedle array patches was explored to further improve the therapeutic effectiveness of the topical treatment. Since the skin of the patients is thickened compared to healthy skin, microneedles offer the possibility of forming microchannels in the stratum corneum, the skin's primary barrier. This allows for an increase in the amount of the active ingredients that penetrate into the skin. The present work is intended to contribute to improving the topical therapy of psoriasis patients. It aims to increase the number of patients who can be effectively treated with topical therapy alone. It also aims to reduce the need for systemic treatments while minimizing the risk of associated side effects.

List of publications

Publication 1

Larissa Carine Pünnel and Dominique Jasmin Lunter, Film-Forming Systems for Dermal Drug Delivery. Pharmaceutics. 2021. 13(7), 932. DOI: 10.3390/pharmaceutics13070932

Publication 2

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Publication 3

Larissa Carine Pünnel, Maria Palmtag, Dominique Jasmin Lunter and Jillian L Perry, Development of 3D printed microneedles of varied needle geometries and lengths, designed to improve the dermal delivery of topically applied psoriasis treatments. European Journal of Pharmaceutics and Biopharmaceutics. 2024. Volume 204, 114523. DOI: 10.1016/j.ejpb.2024.114523

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Larissa Carine Pünnel and Dominique Jasmin Lunter

Larissa Carine Pünnel

Investigation, writing – original draft preparation

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Development of 3D printed microneedles of varied needle geometries and lengths, designed to improve the dermal delivery of topically applied psoriasis treatments

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Larissa Carine Pünnel, Maria Palmtag, Dominique Jasmin Lunter and Jillian J Perry

Larissa Carine Pünnel

Writing – original draft, Methodology, Investigation, Conceptualization.

Maria Palmtag

Methodology, Investigation.

Dominique Jasmin Lunter

Conceptualization, methodology, writing - review and editing, supervision

Jillian L Perry

Writing – review & editing, Validation, Supervision, Methodology, Investigation, Conceptualization

List of Poster Presentations

Larissa Carine Pünnel and Dominique Jasmin Lunter, Semi solid systems with increased substantivity for the treatment of psoriasis, DPhG Annual Meeting, October 2023 in Tübingen, Germany

Larissa Carine Pünnel and Dominique Jasmin Lunter, Development of a formulation for the treatment of psoriasis with increased substantivity, DPhG International PhD Student & Postdoc Meeting, March 2024 in Marburg, Germany

Larissa Carine Pünnel and Dominique Jasmin Lunter, Development of microneedle patches for the treatment of psoriasis, Skinforum, June 2024 in London, Great Britain

Abbreviation

%	Percent
× g	Multiples of gravity
°C	Degree Celsius
API	Active Pharmaceutical Ingredient
BDP	Betamethasone dipropionate
CA	Calcipotiole
CLIP	Continuous liquid interface production
CO	Castor oil
DAAD	Deutscher Akademischer Austauschdienst
e.g.	Exempli gratia; for example
ESI	Elektronspray-Ionisation
etc.	Et cetera; and other similar things
FFF	Film-forming formulation
g, mg, µg	Gram, milligram, microgram
G’	Dynamic module
G’’	Loss module
h	Hour
HPLC	High Performance Liquid Chromatography
i.e.	Id est; that is
LC	Liquid chromatography
log	Decadic logarithm
LOD	Limit of Detection
LOQ	Limit of Quantification
m	Meter

MAP	Microarray patches
MCT	Medium-chain triglycerides
min	Minute
mL, µL	Milliliter, microliter
mm, µm, nm	Millimeter, micrometer, nanometer
MS	Mass spectroscopy
n	Number of experiments
Pa	Pascal
PBS	Phosphate buffered saline
PEG	Polyethylene glycol
Ph. Eur.	European Pharmacopoeia
PPG	Polypropylene glycol
R^2	Coefficient of determination
rpm	Revolutions per Minute
s	Second
SC	Stratum Corneum
SD	Standard Deviation
TEWL	Transepidermal water loss
UV	Ultraviolet Radiation
v	Volume
λ	Wavelength

1. Introduction

1.1 Psoriasis

Psoriasis is a chronic inflammatory skin disease that cannot be cured.[1] Approximately 1-3 % of adults worldwide are affected by the disease, most of whom suffer from the subtype plaque psoriasis.[2]

Plaques can occur on all areas of the patient's skin, but they commonly affect the scalp, mucous membranes and fingernails. The affected skin areas are reddened and the skin surface is covered with thick, silvery scales. The fingernails are generally thickened, pitted or ridged. As the patients skin is particularly dry, they suffer from itching and soreness along with cosmetic impairments. Psoriasis can significantly impact a person's quality of life, causing physical discomfort, emotional distress and social isolation.[2]

The pathogenesis of psoriasis is not yet fully understood. Macrophages, dendritic cells and natural killer T cells release the cytokines tumor necrosis factor alpha (TNF-alpha), interferon alpha and gamma as well as interleukin 1 beta.[3] Myeloid dendritic cells then release interleukin 12 and 23. Interferon 23 ensures the proliferation of T helper 17 cells and T helper 22 cells, which in turn secrete interleukin 17 and 22 as well as TNF-alpha.[4]

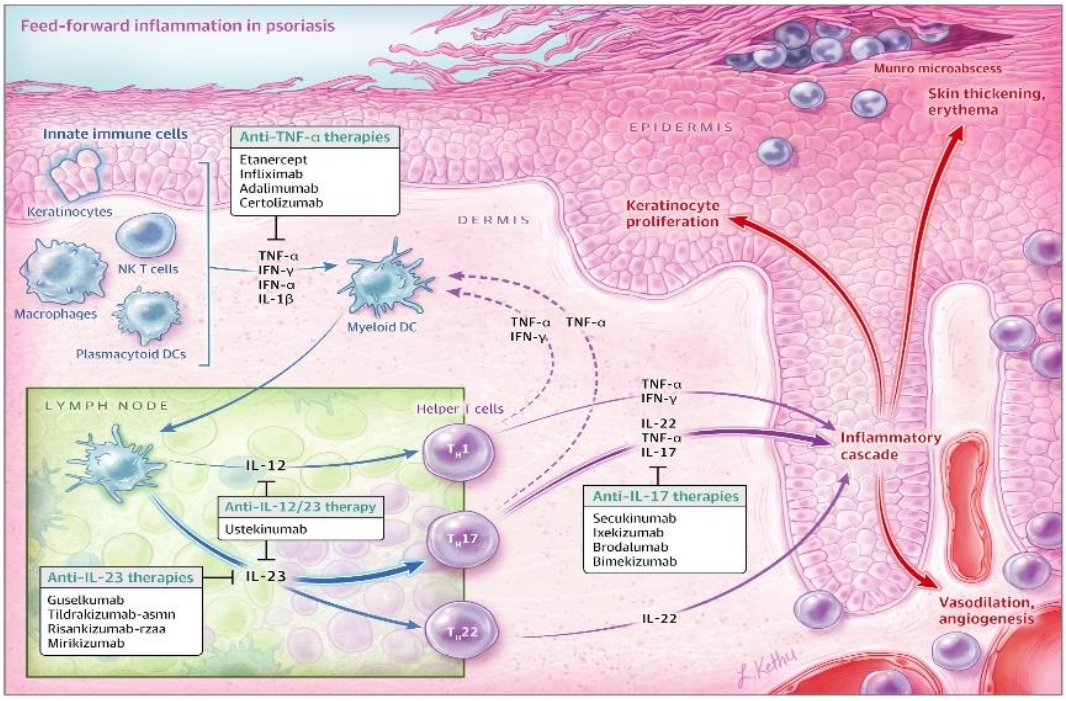


Figure 1: Pathophysiology of Psoriasis (Copyright 2020 American Medical Association. All Rights Reserved)[4]

As shown in figure 1, these cytokines cause inflammation of the dermis, which leads to hyperproliferation of the keratinocytes. As the new, rapidly formed skin cells push the existing cells upwards, the normal transport period of 28 days, during which a cell moves from the stratum basale to the stratum corneum, is shortened.[5] Due to this shortening, the skin cells cannot differentiate properly and appear silvery white on the skin surface. The skin barrier is disturbed by the improperly differentiated cells. This leads to increased trans epidermal water loss and increased penetration of germs. This in turn activates the synthesis of further cytokines and T lymphocytes, which intensify the inflammation. Therefore, a topical therapy is essential for the treatment of psoriasis. Firstly, the target site can be directly addressed with topical formulations, thus avoiding systemic side effects such as immunosuppression. Secondly, by selecting an appropriate base, the lipid matrix of the skin, and therefore the skin barrier, can be strengthened. This leads to fewer inflammatory reactions in the skin.

1.2 Skin as an Application Site for Drugs

With approx. 2 square meters, the skin represents a large application site for drug delivery.[6] A formulation that is applied onto the skin surface can be absorbed into the different skin layers allowing it to exert localized effects within each layer. In case of deeper penetration into the dermis, the drug enters the bloodstream, which leads to a systemic effect.[7] Using the skin as an application site for drugs offers several advantages. Dermal diseases, like chronic inflammatory diseases or bacterial and fungal infections can be treated without systemic side effects. Drugs that lose their effect metabolically due to the first-pass effect when administered orally can still be used.[8] The structure of the skin, penetration pathways as well as dermal and transdermal systems are explained in 2.1.

1.3 Ex-Vivo and In-Vitro Models for Penetration and Permeation

In order to investigate the penetration and permeation of drugs, in-vitro, ex-vivo and in-vivo studies can be performed. As the testing of topical and transdermal formulations in animals and humans only takes place in late phases of drug product development, in-vitro and ex-vivo models are used for earlier development stages.

All models used for in-vitro experiments attempt to mimic the physiological conditions in a controlled environment of the relevant tissue.[9] To simulate human skin there are two-dimensional (2D) and three-dimensional (3D) in vitro cell culture assays available, which

simulate all three skin layers. Additionally, 3D bioprinting technology enables the creation of complete skin models.[10-12]

Ex-vivo experiments are performed on tissues from a living organism but outside of their body in a controlled environment. To investigate the penetration of APIs into and through the skin human cadaver skin is most suitable.[13] As this is only available to a limited extent, the skin of animals with a similar structure to human skin can be used.[14] Porcine skin of is suitable for this purpose.[15]

There are various systems for simulating the penetration and permeation of substances into and through the skin. They all consists out of three parts: A skin barrier separates the donor compartment with the formulation from the acceptor compartment that's filled with phosphate buffered saline (PBS). Each active ingredient penetrates and permeates the skin individually. To detect the permeated amount of API the concentration in the acceptor medium can be measured while the skin sample has to be destroyed to measure the penetrated amount of API into the different skin layers.[16] The gold standard is the Franz diffusion cell that is shown in figure 2. The entire cell is made out of glass and the skin is located between the donor and the acceptor compartment. The acceptor compartment is usually filled with PBS buffer that is stirred using a magnetic stirrer and tempered to 32 °C. On the side of the donor compartment a sampling arm is applied out of which a defined amount of buffer can be taken. Different diffusion chamber models are the Parallel Artificial Membrane Permeation Assay (PAMPA) and skin-on-a-chip models.[17, 18]

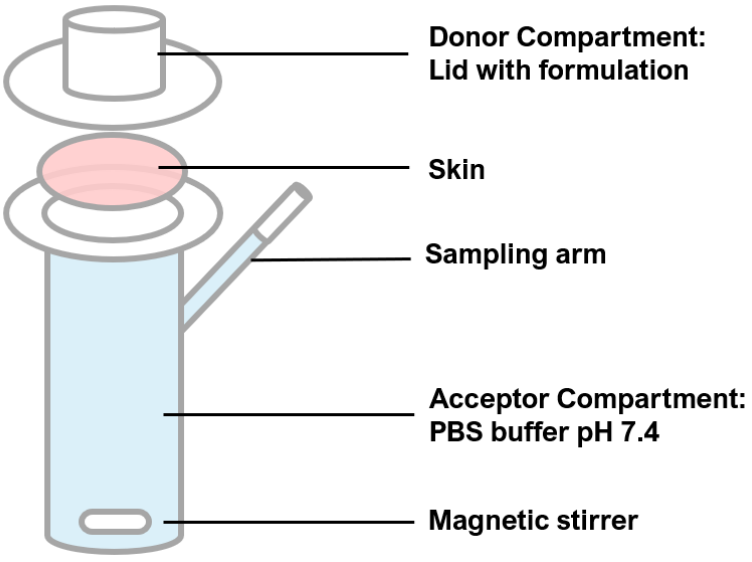


Figure 2: Franz diffusion cell

1.4 Semisolid Dosage Forms for Dermal Application

According to the European Pharmacopoeia (Ph. Eur.) semisolid preparation for cutaneous application are described as "preparations intended for application to the skin to deliver active substances for local or systemic effect, or to exert an emollient or protective action".[19] The Ph. Eur. divides these into 6 categories, each of which is further subdivided according to its properties as shown in figure 3.

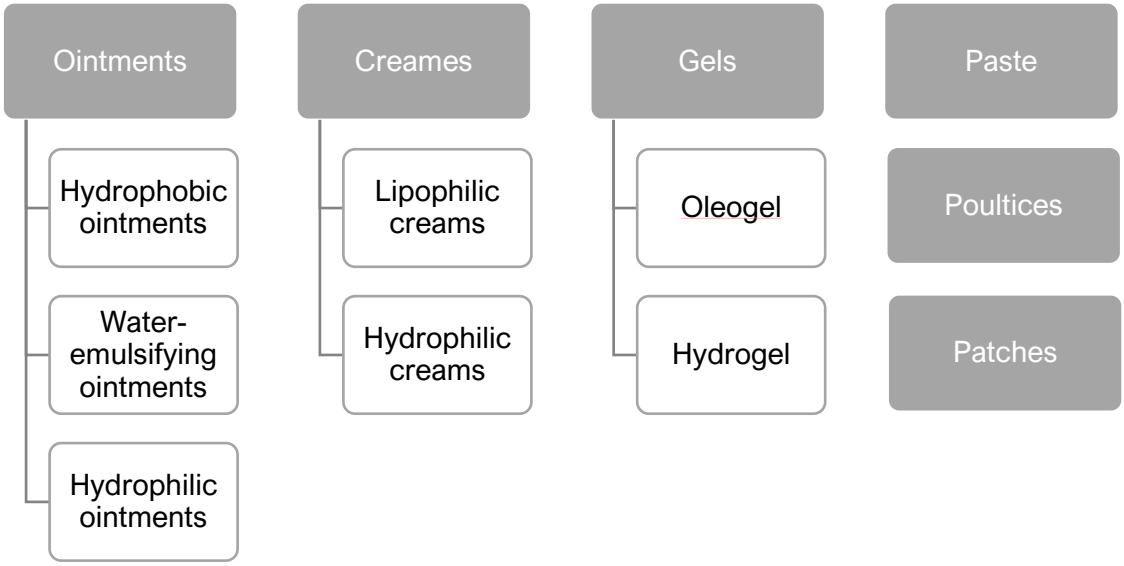


Figure 3: Overview semi-solid dosage forms

They always consist of a base made up of one or more excipients in which an API can be dissolved or dispersed.[20, 21]

The decision as to which type of semi-solid preparation is selected for the treatment of a disease depends on the condition of the skin, the type of disease as well as on the active ingredients used. If the active ingredient is to penetrate the skin or through the skin, it must dissolve sufficiently in the preparation and strive to leave the preparation, as only dissolved active ingredients can penetrate the skin.[21]

1.4.1 Ointments

Ointments are single phase systems. They can be divided in three categories. Hydrophilic ointments are based on polyethylene glycol (PEG) with different chain lengths. Hydrophobic ointments are based on liquid, semi solid and solid lipophilic substances. For the treatment of psoriasis, ointments based on petroleum jelly are used. By covering the skin with the lipophilic components, less water can evaporate from the skin surface. Water from underlying skin layers collects underneath and loosens the

stratum corneum. The skin is thus hydrated and active ingredients can penetrate the skin more easily.[22, 23]

The ointments Daivobet®, Enstilar® and Xamiol® were used in this work. The Daivobet® and the Enstilar® are petroleum jelly and paraffin based in which the active ingredients BDP and CA are dispersed. Polypropylene glycol (PPG) - 11 stearyl ether is used to improve the solubility of CA and the two antioxidants Butylhydroxytoluene and α-Tocopherol are used to protect CA from oxidation. To Enstilar® butane and dimethyl ether are added. This increases the solubility of BDP in the formulation, so that the proportion of dissolved BDP is increased compared to the Daivobet® ointment. .[24, 25] The ointment Xamiol® was also selected for comparison with the developed formulation. It consists of hydrated castor oil and paraffin, as well as PPG-11-stearylether, butylated hydroxy toluol and α-tocopherol.[27]

1.4.2 Creams

Compared to ointments, creams are always two-phase systems consisting of a hydrophilic and a lipophilic phase. The character of the cream is determined by the used emulsifier. The phase in which the emulsifier dissolves better becomes the outer phase of the cream, which determines its properties.[23] Wynzora® cream is the only cream that is available for the treatment of psoriasis. Isopropyl myristate, paraffin and medium-chain triglycerides form the inner lipophilic phase, since macrogol glycerol hydroxystearate is a nonionic emulsifier that forms hydrophilic creams. The outer phase is formed by water, which is gelled due to the use of poloxamer (407) as well as carbomer (45,000-65,000 mPa*s). Trolamine, disodium hydrogen phosphate and sodium dihydrogen phosphate are used to form and stabilize the gel. The used isopropanol evaporates on the skin surface after application and creates a cooling effect. Butylated hydroxy anisole and α-tocopherol are used as antioxidants to protect CA from degradation.[26]

1.4.3 Gels

Gels can be divided into hydrophilic and lipophilic gels. Like ointments, they are single-phase systems and are characterized by a solid gelling agent that forms a three-dimensional network that immobilizes the liquid components.[23]

In this work, an oleogel for the treatment of psoriasis was developed in which the base consists of three different lipophilic components into which two active ingredients with a predominantly lipophilic character are incorporated.

1.5 Substantivity

Substantivity describes how well a formulation remains on the skin surface. The higher the substantivity, the less preparation is removed from the surface through contact with other skin areas or clothing. This quality is especially desirable for topical applications, ensuring they remain effective. Active ingredients need adequate time to penetrate the skin from the formulation, and a consistently thick film for example is crucial for sunscreens to provide their protective effect. The extended dwell time of the formulation means that the application intervals can be extended, which contributes to improved patient compliance.

Another positive effect is that the reduced amount of formulation removed means less risk to the environment. Contamination of other people, environmental damage caused by residues in water and soiling of clothing can be minimized.

One way to enhance substantivity is through film forming formulations. Examples of these formulations and explanations of how to achieve this high substantivity are described in 2.

Since the existing film-forming formulas in the form of aqueous solutions, hydrogels and emulsions are water-based and a high proportion of lipophilic components is required for the treatment of psoriasis, an oleogel with the lipophilic film-forming agent OleoCraft™ MP-30 known from cosmetics was developed.

The developed topical formulation that exhibits increased substantivity compared to conventional in-market products was investigated regarding what percentage of the formulation is removed on contact with a piece of textile and to what extent this differs from in-market products and how the penetrated amount of active ingredient is reduced when the formulation and in-market products are in repeated contact with a piece of textile for three hours.

1.6 OleoCraft™ Film former

Since a formulation for the treatment of psoriasis should have a high proportion of lipophilic components, the film-forming agent used must also have lipophilic properties. The OleoCraft™ film formers are macromolecules made of polyamides and are solid, odorless and almost transparent at room temperature.

When heated above 83 °C, the polymers begin to dissolve in liquid oils. The softening point depends on the type of OleoCraft™. When the uniform mass cools down, the amides form hydrogen bonds with each other, creating a three-dimensional network, which is shown in figure 4. The used oil is immobilized in this framework and a gel is formed. The viscosity depends on the type and quantity of polyamide used.[28]

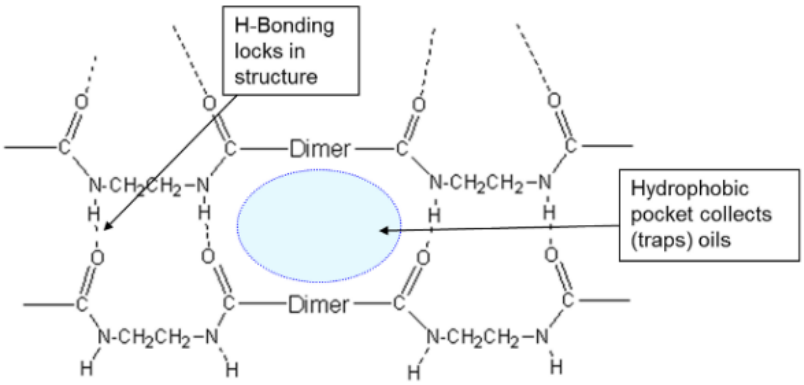


Figure 4: Schematic representation of the gel network.[28]

Different types with varying molecular weights are available on the market, each compatible with oils of different properties and used in a concentration ranging from 1 % to 30 %. As can be seen in Table 1, compatibility with polar substances increases with molecular weight. Since the skin of psoriasis patients is very dry, the preparation should be kept lipophilic, and the amount of film-forming agent used should be as low as possible to avoid to possible irritation.[28]

Table 1: Overview of properties of the OleoCraft™ film-formers.

Product	Molecular Weight	Oil Polarity Compatibility
OleoCraft™ LP-20	~4,500 Da	Low to medium polarity oils
OleoCraft™ MP-30	~20,000 Da	Medium to high polarity oils
OleoCraft™ MP-32	~30,000 Da	Medium to high polarity oils
OleoCraft™ HP-31	~11,500 Da	High polarity oils and water & glycol systems

Since these film formers are suitable for topical formulations, for this work a lipophilic preparation was to be developed that has increased substantivity and is still easy to spread.[29] The Oleocraft™ film former MP-30 was selected and used in a concentration of 7%. A mixture of equal parts castor oil and medium-chain triglycerides was chosen as the oil phase.

1.7 Betamethasone dipropionate

The cortisone derivative BDP has emerged as the gold standard in combination with CA for the treatment of psoriasis. Due to the esterification at positions 17 and 21, it has a log P value of 3.96, making it significantly more lipophilic than hydrocortisone, for example, which has a log P value of 1.6. [30] [31, 32]

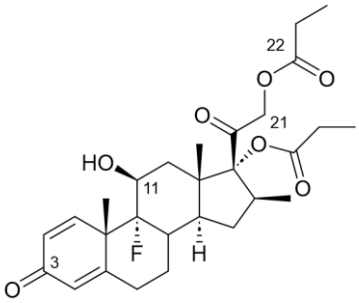


Figure 5: Structure of betamethasone dipropionate

It is only used topically, as both its oral bioavailability and inhalation efficacy are poor compared to other cortisone derivatives.[33, 34] The two esters at positions 17 and 21 make the use of BDP in aqueous preparations impossible. Under acidic catalysis, the acyl group of the ester at position 17 rearranges, while at higher pH values both esters saponify, which is why the use of hydrophobic preparations is recommended.[35, 36]

As a derivate of cortisone BDP binds to the glucocorticoid receptor in the cytoplasm. After binding, the ligand-receptor-complex passes from the cytosol into the cell nucleus where it binds the glucocorticoid response element of specific DNA sequences.[37] Dimerization of two receptors occurs, which leads to the transcription of anti-inflammatory genes. Nuclear factor kappa B is inactivated. As a result, inflammatory mediators such as Cyclooxygenase-2, inducible nitric oxide synthase, intercellular adhesion molecules, vascular cell adhesion molecules and cytokines are no longer transcribed. [33, 38]

At the same time, the reduced expression of inflammatory mediators also leads to reduced expression of fibroblasts and collagen, resulting in skin atrophy.

1.8 Calcipotriole

CA is a synthetic analog of vitamin D3. CA includes a hydroxyl group at the 1α-position and a double bond between the 22nd and 23rd carbon atoms.[39] CA binds to the vitamin D receptor in keratinocytes of the skin. After binding the receptor, the complex translocates into the nucleus to bind to the vitamin D response element. It reduces the

production of pro-inflammatory cytokines, such as interleukin-2 and interferon-gamma and the hyperproliferation of keratinocytes.[40, 41]

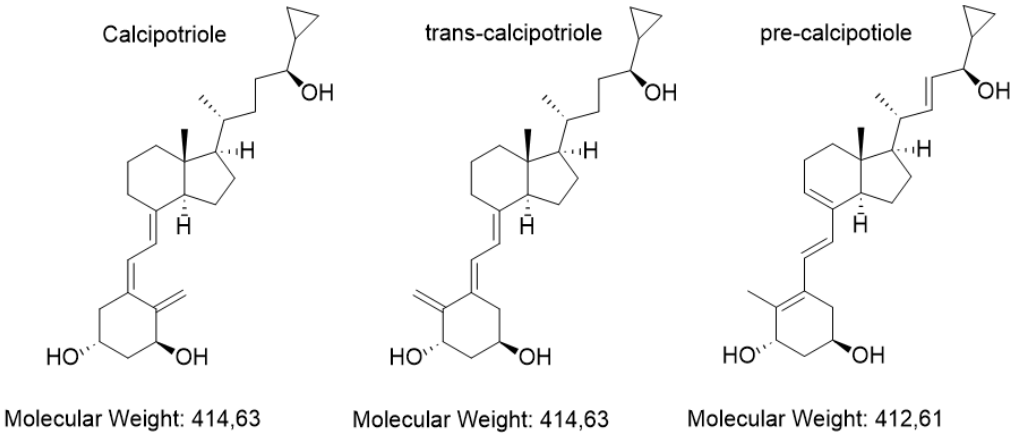


Figure 6: Calcipotriole and the 2 known degradation products trans-calcipotriole and pre-calcipotriole.[39]

With a log P value of 4.6, the drug dissolves better in lipophilic substances, which makes it suitable for use in ointments and oleogels.[42] Another reason not to use the drug in aqueous preparations is its instability. When exposed to light, heat and several solvents CA rearranges to structures like trans- or pre-calcipotiole that are shown in figure 6.[36, 39, 43]

1.9 Microneedle array patches

Microneedle array patches (MAP) can be used to improve or enable the penetration of APIs into the skin for dermal or transdermal use.[44] These consist of a patch with needles ranging in length from 100 µm to 1 mm in different shapes, lengths and materials.[45] The microneedles puncture the stratum corneum that represents the largest barrier for active ingredients to penetrate first, followed by the epidermis and dermis. Depending on the shape of the needles and the applied force. The microneedles achieve skin penetration at a depth of 50-80 % of their overall length. With a length of 1 mm or less, the MAPs are short enough to not reach the pain receptors in the dermis enabling a painless application and use.[46]

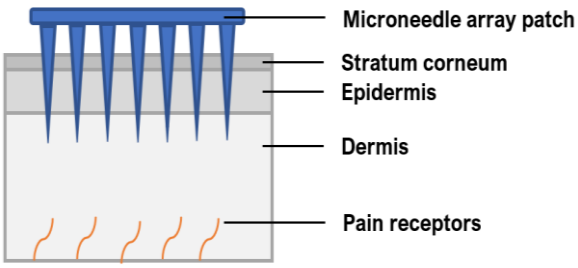


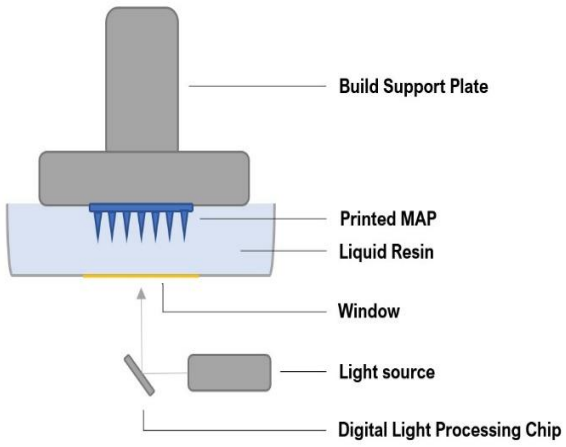
Figure 7: Schematic representation of a MAP after penetration into the skin

The amount of API that penetrates the skin out of a semi-solid formulation increases due to the formed microchannels in the stratum corneum. Since the stratum corneum of psoriasis patients in thickened compared to healthy skin, MAPs have the potential to increase the effectiveness of topical treatment.

MAPs are made from biocompatible materials like polymers, silicones, metals or ceramics. They can be designed as solid or hollow needles. For therapeutic use it is possible to coat MAPs or to design them out of a biodegradable material that releases a therapeutic dose of an API. Regardless of the design, the needles must be able to withstand penetration into the skin and must be able to be removed without causing long-term damage to the skin. They can be produced using several techniques like 3D printing.

1.9.1 Continuous liquid interface production

Continuous liquid interface production (CLIP) technology is a method of 3D printing to manufacture small objects with a smooth surface out of a photopolymer resin and was therefore chosen to manufacture the MAPs that were designed and tested in this study.



The liquid resin from which the product is printed is stored in a reservoir. Through the window in the bottom of the reservoir, ultraviolet light reaches the resin precisely and causes it to solidify on the underside of the build support plate. After printing a layer, the die moves up a little so that resin can flow under the stamp and the next layer is printed. In contrast to stereolithography, an entire layer of the object can be printed, which makes the printing process significantly faster.[47]

Figure 8: Schematic representation of the CLIP printing.

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2. Film-Forming Systems for Dermal Drug Delivery

Larissa Carine Pünnel, Dominique Jasmin Lunter

Department of Pharmaceutical Technology, Eberhard Karls University, Auf der Morgenstelle 8, 72076 Tübingen

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2.1 Abstract

Film-forming formulations represent a novel form of sustained release dermatic products. They are applied to the skin as a liquid or semi-solid preparation. By evaporation of the volatile solvent on the skin, the polymer contained in the formulation forms a solid film. Various film-forming formulations were tested for their water and abrasion resistance and compared with conventional semi-solid formulations. Penetration and permeation studies of the formulations indicate a potential utility as transdermal therapeutic systems. They can be used as an alternative to patch systems to administer a variety of drugs in a topical way and may provide sustained release characteristics.

Keywords:
film-forming system; topical drug delivery; polymeric formulation

2.2 Introduction

*2.2.*1 Skin

The skin is the largest organ of the human body.[1] It acts as a protective barrier against external influences such as ultraviolet radiation, chemical and physical insults, attacks by harmful microorganisms, and mechanical irritation. Furthermore, the skin regulates physiological parameters of the body by providing a barrier against water evaporation and temperature loss.[2]

Skin diseases such as infections triggered by bacteria or viruses, as well as the immunologically caused chronic skin diseases psoriasis, atopic dermatitis, urticaria, or ichthyosis damage the skin barrier. Physiological functions can no longer be maintained, increasing the risk of further infection. Symptoms such as pain, soreness, and wetness affect the patients' quality of life.[3]

2.2.2 Skin Anatomy

Human skin consists of two layers, the epidermis and the dermis. The epidermis can be divided into four layers, with the stratum corneum being on top, followed by the stratum granulosum, stratum spinosum, and stratum basale.[4]

The stratum corneum plays the most decisive part in skin penetration. It consists of dead corneocytes embedded in a crystalline lamellar lipid matrix. In contrast to the living keratinocytes, the already dead corneocytes here have a solid protein envelope that prevents the absorption of substances. This envelope has a hook-like structure which enables the corneocytes to interlock with each other. Between the surfaces of the corneocytes are the corneodesmosomes, proteins that connect the individual corneocytes to each other. The structure of corneocytes, connected via corneodesmosomes, is already formed in deeper layers of the epidermis and is supplemented by further proteins at the transition to the stratum corneum in order to strengthen the mechanical stability of this skin layer.[5]

Between the stratum corneum and the stratum granulosum, there are tight junctions that separate the stratum corneum from the lower layers of the epidermis.[6]

The stratum granulosum and stratum spinosum mainly protect the lower skin layers from water loss and are the site of differentiation of the corneocytes. The lowest layer of the epidermis is the stratum basale. The keratinocytes are produced from the stem cells contained there.[7] The transit time from their formation in the stratum basale to the stratum corneum is 14 days. The complete renewal cycle takes 28 days.[8]

The dermis beneath the epidermis ensures the flexibility of the skin and temperature maintenance of the body. It consists mainly of collagen fibres interspersed with elastic fibres which are surrounded by a matrix of proteoglycans and glycoproteins. Blood vessels, lymphatic channels, and sensory nerves run through the dermis.[8] The dermis and subcutis underneath are not relevant for the penetration of substances for dermal therapy. In systemic therapy with transdermal systems, however, drugs must be able to penetrate to the blood vessels in the dermis and subcutis.

2.2.3 Biochemistry of the Stratum Corneum

The surrounding lipid matrix consists equally of ceramides with a chain length of 16 to 33 carbons, cholesterol derivatives, and free fatty acids, which predominantly have chain lengths of 22 or 24 carbon atoms. The lipids are arranged in a lamellar structure in which

the lipophilic parts of the individual components are arranged parallel to one another, and the hydrophilic head groups of the ceramides, cholesterol, and free fatty acids are directed towards one another. The non-covalent interactions of the head groups thus create lipophilic and hydrophilic areas in the stratum corneum from which the lipid bilayer results. Investigations of the composition of the individual components showed that cholesterol derivatives and ceramides have a hexagonal structure in a 1:1 ratio. The addition of free fatty acids results in a shift to orthorhombic packing. The packing density is increased, which leads to the deduction that a balanced composition of the ingredients is essential for an intact skin barrier.[7] Substances can be transported intercellularly, transcellularly, or on a corneodesmosomal pathway through the stratum corneum.[4] The mechanism of the penetration will be addressed in a subsequent part of this review.

*2.2.*4 Transdermal Transport

Penetration into the stratum corneum is the limiting factor for the amount of drug that can be absorbed.[9] Drugs can pass through the stratum corneum by trans epidermal, trans follicular, or trans glandular routes. Depending on the drug and formulation, they penetrate the skin to different depths as shown in Figure 1.[10] Whether and how much of a substance penetrates the skin depends on several factors. The properties of the substance to be applied to the skin and the formulation in which it is integrated have the greatest influence on penetration. The condition of the skin, i.e., the skin area and the condition of the skin barrier, which in turn depend on age and individual living conditions, also influences penetration.[2]

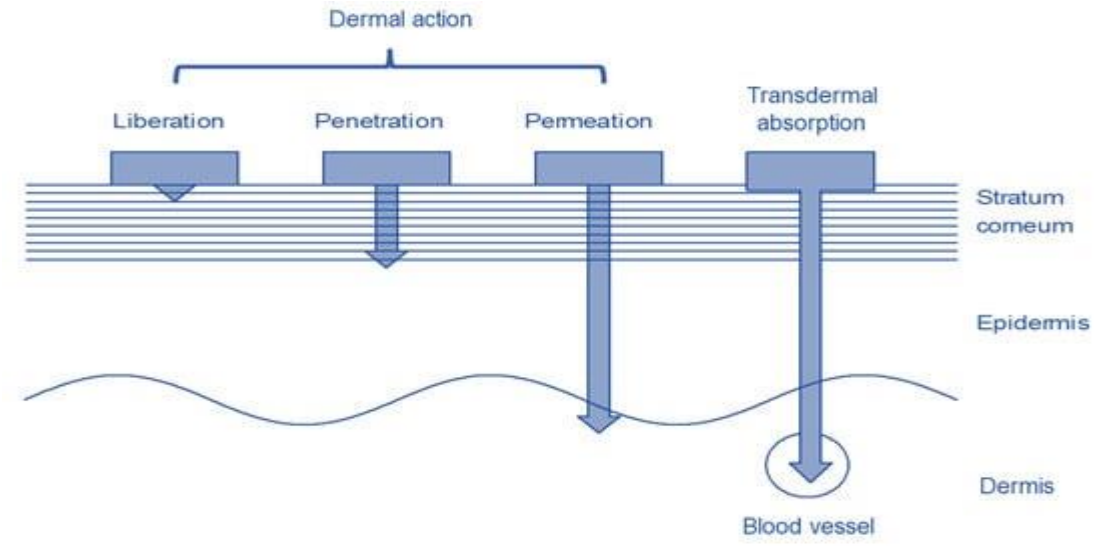

Figure 1. Structure of the skin with the different penetration depths of the dermal and transdermal systems.

Three different penetration mechanisms can be distinguished in the penetration of drugs via trans epidermal route. In the transcellular pathway, the drug alternately penetrates the hydrophilic intracellular space, the corneocytes with their protein envelope and the corneodesmosomes, and the lipophilic intercellular space, the lipophilic matrix. This multiple portioning between hydrophilic and lipophilic matrix is very unlikely.

Via the intercellular route, drugs penetrate by crossing the stratum corneum through the lipophilic matrix. Substances that have a predominantly lipophilic character usually penetrate quickly and completely into the lipophilic matrix. As the underlying layers of the dermis are of a less lipophilic character, the penetration of highly lipophilic substances from the stratum corneum into these skin layers is usually slow, so that a reservoir of the substance is formed in the stratum corneum.[11] Drugs with a dominating hydrophilic character can also penetrate via the intercellular route along the hydrated hydrophilic head groups of the stratum corneum lipids. Their penetration by this route may be increased by penetration enhancers or occlusion. Ingredients such as fatty acids, surfactants, and terpenes can be used as penetration enhancers for transdermal transport, while monoglycerides and oxazolidinones are used increasingly for dermal use. Most of them increase the penetration by disruption of the lamellar lipid structure in the stratum corneum. However, the substances used to improve penetration have a disadvantage in that they can permanently disrupt the skin barrier, which leads to skin irritations. The allergenic potential is also increased. These risks can be avoided by combining different enhancers.[12] Another way to improve the penetration of hydrophilic substances is skin occlusion. Formulations with a high lipophilic content reduce the evaporation of water from the skin. The drug can dissolve in the hydrated stratum corneum.[13] The resulting swelling of the skin is often perceived as unpleasant by patients, so other ways of improving penetration should be preferred. Studies have proven that skin occlusion and disruption of the skin barrier can increase the penetration for many hydrophilic drugs, but not for all.[7] Other hydrophilic drugs penetrate via the corneodesmosomal route, which represents the third penetration route of the trans epidermal route. Hydrophilic substances penetrate the stratum corneum along the hydrophilic protein envelope of the corneocytes and the protein-rich corneodesmosomes. Due to the swelling of the corneocytes during occlusion, caused by water retention and disturbance of the skin barrier, these corneodesmosomal bonds can break.[6] Therefore, if a hydrophilic drug penetrates via the corneodesmosomal pathway and not via the intracellular pathway, penetration can be reduced by occlusion or penetration enhancers.[6]

In addition to the trans epidermal route, lipophilic drugs in particular penetrate well into deeper skin layers via the trans follicular route. Penetration is rapid as, unlike the trans epidermal route, fewer cell layers must be overcome. However, the amount of active ingredient that penetrates is strongly dependent on the amount and character of the hair glands, so this route depends strongly on individual factors.[14]

The glandular route can be neglected, as the majority of the drug is transported out wards by secretion which means that this route cannot be considered for reliable penetration. The penetration pathways described have been researched using healthy skin. In the case of chronic skin diseases such as psoriasis, atopic dermatitis, chronic dermatitis, and ichthyosis, this skin barrier is disturbed, which makes the development of formulations for therapy challenging. The penetration of substances cannot be adequately predicted.

2.2.5 Dermal Systems

Dermal systems are usually designed to only achieve a local effect. They are used for the therapy of acute skin infections and chronic skin diseases. Usually, a liquid or semi-solid formulation is applied at regular intervals. The formulation should be designed in such a way that the active ingredient contained can penetrate into the epidermis and develop its effect. To avoid a systemic effect, penetration into the dermis should be kept to a minimum. Especially in the case of drugs such as antibiotics and immunosuppressants such as cortisone, a sufficiently high therapeutic quantity can be achieved by dermal application without systemically burdening the organism with the drugs. The risk-benefit profile can be influenced positively.

Conventional topical preparations are easily washed or rubbed off. Thus, the treatment is often inadequate as the active ingredient is not present in the epidermis in sufficient and consistent quantities. Using a higher concentration of the drug is not a solution for this drawback, as the concentration fluctuations would only be amplified and the probability of active substance penetrating into the dermis would increase. Formulations exhibiting high substantivity provide a more elegant solution. The substantivity is defined as the capacity of the active substance to be kept at the site of action, on the surface or as a reservoir in the stratum corneum.

Using patches and plasters for dermal treatment of skin diseases may provide an alternative. They create a physical barrier between the formulation and the environment which protects the formulation from erosion. This physical barrier usually leads to occlusion of the skin, which, in addition to the affections of unappealing appearance and

negative wearing comfort, is detrimental to patient compliance. In most cases, the patches cannot be cut to the required size, which often makes them unsuitable for individualised therapy. Skin damage can occur when the patches are pulled off, which makes them unsuitable for use in diseases with a disturbed skin barrier. Allergic reactions to the matrix adhesives are another problem, so these systems are not optimal either.[15]

2.2.6 Transdermal Systems

In transdermal systems, an active ingredient incorporated into a liquid or semi-solid formulation is applied to the skin, or the drug is delivered via a patch. In contrast to dermal systems, the aim is to deliver the active to the systemic circulation. Compared to other forms of delivery, transdermal systems offer many advantages. They are one way to avoid first pass effect. Food effects or intestinal absorption problems are no issue. Compared to injections or intravenous applications during the administration of drugs, the skin is not injured by needles or incisions.[16]

With patches, which are the most common treatment, the sustained release of the drug is usually easy to realise. The drug is embedded in its formulation in the membrane of the patch and may be released through a control membrane or is controlled by diffusion in the adhesive. Patches are usually easy to apply, as they are simply administered at correct time intervals to uninjured, hairless areas of the body surface. When properly applied, the drug release is continuous so that the plasma level is constant. The active ingredient is well protected from external influences in the formulation by the backing layer. In the case of insufficient penetration, in addition to penetration enhancers, systems such as microneedles or iontophoresis can also be used to improve penetration. The disadvantages of patches for transdermal use are the same as in the use of dermal delivery, namely the cosmetic aspect. Currently, mainly opiates and hormones are applied transdermally. However, transdermal application is an option for a lot of drugs with a low therapeutic range and drugs with stability problems.[17]

2.3 Film-Forming Systems

As described, the main problem with dermal and transdermal application of drugs via liquid and semi-solid formulations is the washing and rubbing off of the formulations, so that the desired therapeutic effect cannot be achieved. For this reason, very few semi-solid formulations are found in transdermal use. The idea behind the development of film-forming formulations is to develop formulations with increased substantivity against

mechanical and water-based influences and improvement of the cosmetic properties, and thus patients' compliance. As dosage forms, sprays, gels, or emulsions may be formulated.[18,19]

As shown in Figure 2, the formulation is applied evenly to the skin surface. After the application of the solution or gel, the volatile solvent evaporates rapidly and the film-forming polymers interact with one another to form a thin transparent film, which adheres to the skin.[20] Figure 3 shows Raman-microscopic images of a film-forming emulsion applied to the skin. The emulsion is applied in a liquid state and forms a solid film after drying, similar to the film-forming solutions and gels.

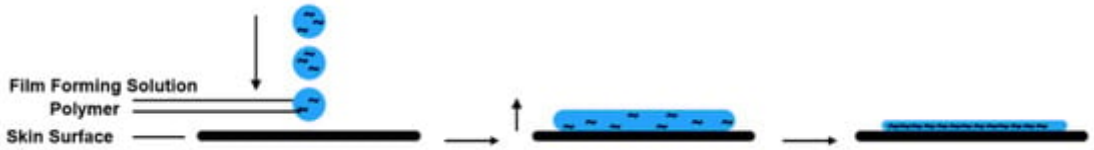

Figure 2. Mechanism of film forming.

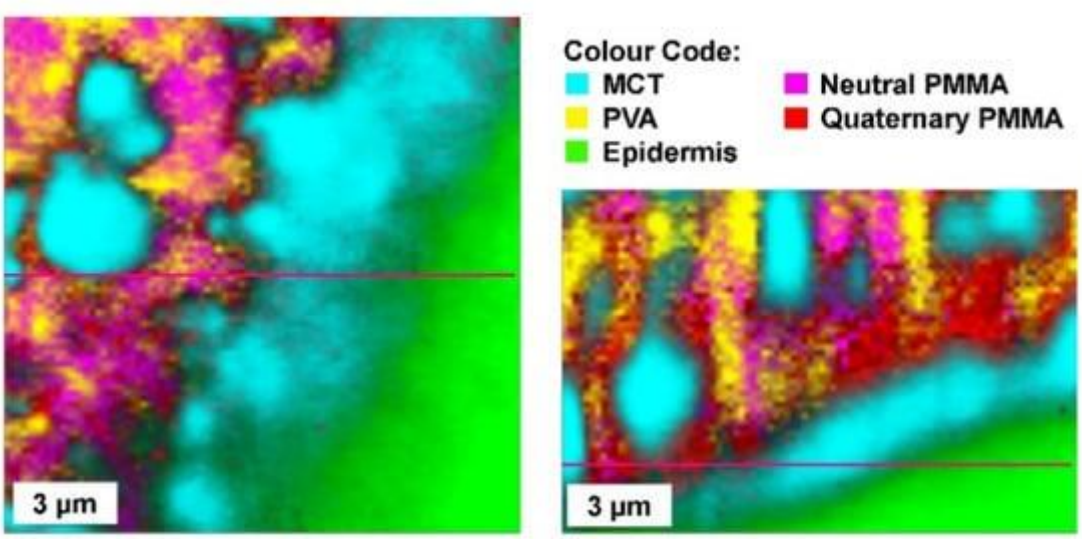

Figure 3. Film-forming emulsion, the oil droplets are embedded in a polymer matrix made of PVA and Eudragit NE (neutral PMMA (Polymethylmethacrylate) and Eudragit RS (quarternatry PMMA) for controlled drug release.

In addition to the volatile vehicle and the film-forming agent, the film-forming systems contain the active ingredient and usually a plasticiser and/or penetration enhancer. These non-volatile components ensure that the dry film does not have any brittle properties but adheres to the skin and forms a flexible and non-tacky film after the evaporation of the volatile solvent. The resulting film must be such that it forms a homogenic reservoir of

the active ingredient on the skin which has a high substantivity, from which the active ingredient can still penetrate well into the stratum corneum.[21] Due to enhanced substantivity, the formulation can also be used as a transdermal system as the correct dose can be delivered reliably. The formed film must adhere firmly to the skin and must not wash or rub off. The drug can have its reservoir in the formulation itself, and penetrate slowly into the stratum corneum, or pass directly from the formulation into the stratum corneum and form its reservoir there. In the latter case, the formed film serves as protection.

This review deals with film-forming formulations containing active ingredients. In the field of semi-solid preparations, gels are listed as film-forming formulations. In the area of liquid preparations, emulsions and solutions, as well as so-called patch-no-patch systems, are discussed. Nano-formulations or formulation for wound healing are not addressed, as dedicated reviews on these topics exist. All formulations mainly consist of the components listed in 2.3.1.1–2.3.1.4. The structure is shown in Figure 4. The film formation of the individual formulation results from the polymers it contains. When the solvent evaporates, the polymer chains interact with one another, and are able to form a solid polymer matrix on the skin surface with a high substantivity that forms a reservoir for the drug. The drug penetrates from the film into the skin to exert its effects locally or systemically.

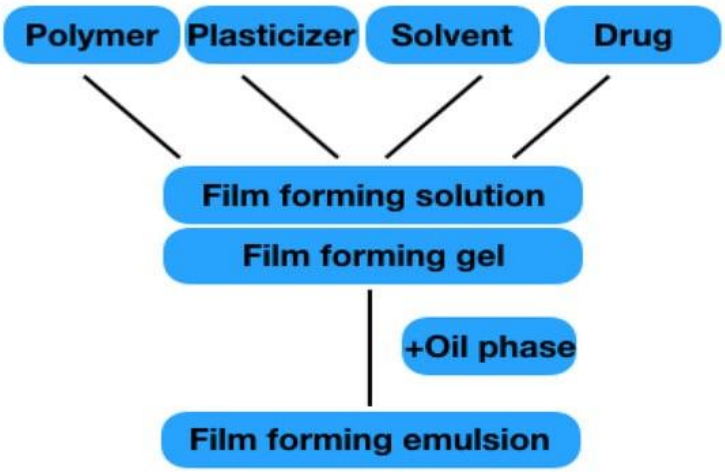

Figure 4. Illustration of different film-forming solution/gel.

Another type of film formation is the application of an oily formulation that forms an occlusive film on the skin. These formulations are not covered in this review.

2.3.1 Components of Film-Forming Systems

2.3.1. Drug

Regardless of whether the drug is meant to be used in topical or transdermal application, it must be able to penetrate well into the stratum corneum. Drugs with a predominantly lipophilic character usually penetrate the skin better than hydrophilic drugs, as the matrix of the stratum corneum also has a lipophilic character. The distribution coefficient log P is ideally located, intermediate between 1 and 3.[22,23] The molecular weight plays a decisive role here, as small molecules exhibit a higher velocity of diffusion. The molecular weight should be lower than 1000 Da [22], ideally lower than 500 Da [24]. Another important property of the drug which needs to be taken into consideration during formulation development is the solubility.

If the vehicle of film-forming formulations mainly consists of organic solvent, the drug must be dissolvable in this. Due to the evaporation of the volatile solvent, the drug concentration in the formulation increases. During the evaporation process, the drug must not crystallise, therefore sufficient solubility of the drug in the non-volatile components of the formulation is equally important. Studies show that a formulation with the dissolved drug should have a pH value of 5 to 10.[22] The pH value of the skin itself is around 5, so the pH value of the formulation should also be in this range to avoid skin irritation during application. Mostly, the optimal pH value for penetration is higher than 7. A pH range of 5–10 is considered as a compromise for both.[25,26]

2.3.2. Polymer

The choice of film-forming polymer has the greatest influence on the substantivity of the formulation. Polymers can be used individually or in combination. It is important that they are able to form a flexible, thin, transparent and resistant film. Essentially, a distinction is made between water-soluble and water-insoluble film formers.[27] Water soluble polymers have a hydrophilic character, and most of them are not suitable for substantivity increase in conventional formulations on the skin surface, but ideal for formulations from which the drug quickly penetrates the stratum corneum to form a drug reservoir there. Some, such as methylcellulose, are suitable to increase substantivity of thermo-emulsions by thermo gelation. In film-forming formulations, the aim is to create a drug reservoir in the formulation itself, for which the hydrophilic polymers alone are not suitable.

Water-insoluble polymers form water-resistant films with high substantivity, but are often brittle and inflexible, which makes adhesion to the skin difficult and causes the film-

forming formulations to crumble. To increase the uniformity of the film and its flexibility, plasticisers are usually added to the formulation, or the polymer is combined with a water-soluble polymer.[20] Small polymers with a low molecular weight are usually better suited to film-forming systems. The viscosity of the formulation increases during the solvent evaporation process, even more so for polymers with a high molecular weight. Furthermore, smaller polymers with shorter chain lengths can usually arrange themselves better in space, so that the distance for the interaction of the polymer chains is closer to the ideal state for gel formation. After the evaporation of the volatile solvent, the formed film adheres uniformly to the skin, so that no major differences in film thickness result. This is particularly important for the penetration of the active ingredient into the skin, as the concentration gradient between the film and the stratum corneum is thus also constant over a longer period of time on all areas of the skin.[28]

Polymers with different properties that have been used for preparation of film-forming systems are mentioned in Table 1. In the future, environmental influences will also become increasingly relevant in the choice of film formers, as most insoluble film formers for modified active ingredient release are considered microplastics, which enter wastewater through precipitation, abrasion, and washing. Therefore, starch, alginate, or cellulose-based formulations should be the focus of the development process in the future.[29]

Table 1. Polymers for use in film-forming formulations.[21,30,31,32]

Polymer	**Properties**
Polymer	Properties
Carbopol (polyacrylat)	water-soluble, pH sensitive
Chitosan (Poly-D-Glucosamin)	water-soluble at pH < 7
Crosslinked polymer layer XPL	adhesive, elastic
Dermacryl 79 (Carboxylates Ac rylpolymer)	water-insoluble
Ethylcellulose	non-toxic, not irritating, anti-allergic
Eudragit NE (ethylacrylate methylmethacrylate copolymer)	water-insoluble, transparent, elastic, adhesive
Eudragit RL-100 (polymethacrylate polymere)	water-insoluble, transparent, elastic, adhesive

Eudragit RS-100 (polymethacrylate polymere)	water-insoluble, transparent, elastic, adhesive
Eudragit L30D-55 (methacrylate-ethylacrylate-copolymer)	water dispersible at pH 2–3
Hydroxypropyl-beta-cyclodextrin	water-insoluble, increases bioavailability
Hydroxypropylmethylcellulose (HPMC)	water-soluble, non-ionic
Klucel (Hydroxypropyl cellulose)	water-soluble, non-ionic
Macrogol	water-soluble
Methyl cellulose	water-soluble
Poloxamer (polyethylenepolypropylene glycol)	thermoreversible
Plastoid (Butyl methacrylate-methylmethacrylate copolymer)	water-insoluble
Polydimethylsiloxane (PDMS)	water-insoluble, non-toxic
Polyvinyl alcohol (PVA)	water-soluble, adhesive, non-toxic
Polyvinyl pyrrolidine (PVP)	water-soluble, adhesive, increase bioavailability
Quaternary polymethacrylat (QPM)	water-insoluble
Sepineo P600 (acrylamide/sodium acryloldimethyltaurate)	water-insoluble
Silicone	water-soluble, non-occlusive

2.3.3. Solvent

In the first instance, the used solvent must be compatible with the skin, even if the skin barrier is disturbed or injured. It should also not irritate the skin during the evaporation process. The film-forming polymer must be well dispersed or dissolved in the solvent. The polymer film should form within less than one minute after the application of the formulation, so the solvent must evaporate quickly at skin surface temperature. At the same time, this must happen uniformly and slowly enough on skin surface that, despite the resulting increase in viscosity of the formulation, the polymers are still sufficiently mobile for interaction and homogenous film forming. Ethanol and isopropanol are particularly suitable solvents for film-forming formulations. Propylene glycol and isopropyl myristate offer the advantage of additional penetration enhancing properties

but do not evaporate.[21,33,34,35] In Table 2, a selection of solvents and dispersion agents used for film-forming systems are summarised.

Table 2. Volatile solvents for film-forming formulations.[21,36]

Solvent	**Properties**
Benzyl alcohole	lipophilic, organic solvent
Butanol	organic solvent
Ethanol	organic solvent, volatile, hydrophil
Ethylacetat	organic solvent, lipophilic
Isopropanol	organic solvent, volatile, hydrophilic
Isopropyl myristat	organic solvent, lipophilic, penetration enhancer
Polyethylene glycole	hydrophilic, penetration enhancer
Water	hydrophilic

2.3.4. Plasticizer

The main problem during the development of film-forming formulations is the brittle nature of many polymer films. If the film is too rigid, it cannot follow the movements of the skin, especially in application areas such as the elbows.[37] The adherence to skin is then also reduced. A combination of different polymers in one formulation can reduce this problem by taking advantage of different properties of the polymers, but usually the use of plasticizer is necessary.[38] Plasticisers can penetrate between the polymer chains of the film-forming polymer and interact with the functional group. The intermolecular forces between the polymer chains are reduced, meaning that the bonds are weaker and the flexibility of the film is thus increase.[37,39] Studies discovered an ideal plasticizer concentration of 5–20% of the total dry weight.[40,41] A combination of different plasticizers can be also useful. In Table 3, conventional plasticisers used in the development of film-forming formulations are shown, together with the polymers with which they can be combined, according to current studies.[37,39,42,43] The indicated concentration refers to the relative amount with regard to the total dry weight in each case.

Table 3. Plasticizer for film-forming formulations.[37,39,44]

Plasticizer	Properties	Polymer	C in %
Dibutylphthalat	Plasticizer	Eudragit E 100, Ethyl cellulose, Polyvinylpyrollidone, Hydroxypropyl methyl cellulose,	10–40
Glycerol	Plasticizer	Polyvinyl Alcohol, Polyvinylpyrrolidone	10–30
Polyethylenglycol 300 or 400	Plasticizer	Hydroxypropyl cellulose, Eudragit E 100, Carbopol,	5
Polysorbat 80	Non-ionic solubilizer, plasticizer, emulsifier, co-emulsifier	Cellulose Acetate	20–50
Propylene glycol	Polymeric solubilizer, plasticizer	Polyvinyl Alcohol, Polyvinylpyrrolidone, Eudragit L100, Hydroxypropyl methyl cellulose, Ethylcellulose, Carboxy methyl cellulose	5–50
Sorbitol	Plasticizer	Polyvinyl Alcohol, Chitosan	2–20
Triacetin	Versatile water or oil miscible solvent. plasticizer	Eudragit E 100	1.43 – 5.48
Triethyl citrate	Plasticizer	Hydroxypropyl methyl cellulose, Eudragit RL and NE, Acrylate copolymer, Polyvinylpyrrolidone, Polyvinyl Alcohol	6

2.3.2 Film-Forming Systems on the Market

At present there are several film-forming formulations on the market. These formulations are already approved as medicinal products and are available.

The most successful application of film-forming formulation so far is as wound care products. With the help of the spray plaster, wounds can be completely closed and, if necessary, simultaneously protected against infections by the disinfecting substances contained.[45,46] Some systems containing active ingredients have also received marketing authorisation and are listed in Table 4 with their ingredients. With Lamisil Once®, it has

succeeded for the first time in developing a formulation for the treatment of athlete's foot with only one application. The solution is applied and the terbinafine hydrochloride remains as a depot under the film for up to 13 days after application.[47] Axiron® represents the first film-forming formulation for transdermal use containing the hormone testosterone to be sprayed into the axel cavity as a solution and can be used as an alternative to hormone patches. The available spray patch for wound treatment does not contain an active ingredient and is therefore only suitable for wound protection.

Epinamics developed several film-forming sprays with their Liqui-Patch® technology. Formulations with Rivastigmine, Testosterone, Terbinafine, and Vitamin D3 were developed but are not on the market yet.[48] MedPharm and Crescita Therapeutics also succeeded in developing their film-forming systems Medspray® and Durapeel® and making various drugs available dermally, though market approvals have not yet been granted. The systems all contain a polymer and volatile solvent and are listed in Table 5.[49, 50]

It is striking that all film-forming formulations on the market, and also planned ones, contain lipophilic drugs.[51] A possible explanation might be that release of hydrophilic drugs does not need to be slowed down due to their poorer penetration into the stratum corneum. Or, a hydrophilic drug penetrates the skin very poorly or not at all, making the formulation of a film-forming system for dermal or transdermal application obsolete. However, formulations with a high substantivity can remain on the skin for a long time, which allows a slower penetration of the drugs to achieve a therapeutic effect.

Table 4. Film-forming formulations on the market.[52,53,54,55]

Ingrediencies	**Axiron®**	**Lamisil Once®**	**Hansaplast® Sprühpflaster**
Formulation	Solution	Solution	Solution
Drug	Testosterone	Terbinafine HCl	-
Polymer	Polyvinylpyrrolidone	Poly(acrylamide-co-isooctylacrylat), Hydroxypropylcellulose	Acrylic copolymer, polyurethane polymer
Solvent	Ethanol 96%, Isopropyl alcohol	Ethanol	Ethanol, water, dimethylether

Plasticizer		Medium-chain triglyceride	
other	Octisalate (UV protection)		
Application	Apply once a day to one axilla only	Single application of 2 g solution, drying time 1–2 min	Spray on a thin film of the plaster spray from a distance of 5–10 cm and allow to dry for 1 min.
Company	Eli Lilly	GlaxoSmithKlineConsumer Healthcare	Beiersdorf

Table 5. Film-forming systems available on market.[48,49,50]

Company	**Product**	**Formulation**
Epinamics	Liqui-Patch®	Film-forming spray
MedPharm	MedSpray®	Film-forming spray
Crescita Therapeutics™	Durapeel	Film-forming emulsion

2.4 Development and Investigation of Film-Forming Systems

Various groups are working on the development of film-forming formulations with different drugs. The topic is especially interesting for the development of sustained-release dermal products. In recent years, various excipients have been compared and tested for their suitability. The development and evaluation of suitable test systems for the comparison of film-forming formulations is indispensable for their development.

The properties of the formulations with regard to drying time, film thickness, film weight, rheological studies, adherence and spreading, stickiness, water vapor permeability, mechanical properties, and film homogeneity must be suitable and compared with one another. The developed formulations should be free of toxic or allergenic ingredients.[56] In case of dermal and transdermal systems, the penetration and permeation of the systems must be investigated.[35] Today, only a few film-forming formulations are on the market. For many drugs for which a dermal or transdermal application option would be useful, film-forming formulations are not yet available. A large number of polymers are still available for the development of new systems. Film-forming systems that have been developed recently will be described in the following chapters.

2.4.1 Film-Forming Solutions

Film-forming solutions are the most common type of film-forming systems. As already described, the solutions mainly consist of a volatile solvent in which film-forming polymers are dissolved or dispersed, together with plasticisers, the drug, and other excipients. The evaporation of the volatile solvent creates a polymer film on skin surface.

Schroeder et al. prepared film-forming solutions by dispersing or dissolving 14 different film-forming polymers with one plasticiser each, triethyl citrate, triacetin, or dibutyl phthalate in ethanol, water, or silicone oil. The film-forming solutions were evaluated for their drying time, cosmetic attractiveness, outward stickiness, integrity on skin, and viscosity. Some dried films were also tested for their mechanical properties such as tensile strength, elongation at break, and water vapor permeability. The developed testing methods are adequate for in vitro investigation. Although, the results cannot be transferred to in vivo, the formulations can still be used as basis for further development of film-forming systems.[57]

Edwards et al. investigated polymethacrylate copolymers for their suitability for the transdermal application of drugs using film-forming systems. For this purpose, the group prepared aerosol sprays with methylphenidate and polymethacrylate copolymers and investigated the influence of the solubility of the drug in the preparations on permeation. They were able to establish differential scanning calorimetry for solubility determination. Eudragit E proved to be the most suitable for the development of formulations, as methylphenidate did not crystallise as much here.[58]

For further investigation, Gravie-Cook et al. were able to show that the choice of polymer and excipients influence the release from the formulation through interaction with the active ingredient by using Raman spectroscopy and atomic force spectroscopy. Thus, the release of betamethasone from a film-forming solution with hydrophobic polymethacrylate copolymer and medium chain triglycerides was superior to the formulation with hydrophilic polymethacrylate copolymer.[59,60]

Misra et al. were one of the first to develop a liquid film-forming solution for the transdermal application of testosterone. For this purpose, testosterone was dissolved in isopropyl alcohol. As film-forming polymers, polyvinyl alcohol and polyvinylpyrrolidone were used in different concentrations. Liquid paraffin and Polysorbate 20 were added. The ex vivo permeation experiments proved a sustained release of the testosterone from the polymer film after a burst release at the beginning. The retarded effect increased with

increasing polymer concentration.[61] The approach of transdermal application of hormone derivatives through film-forming solutions was taken up by Schroeder et al., who developed further formulations for the application of ethinyl oestradiol.[62]

One application area of film-forming solutions is the dermal application of anti-infectives. Antiseptics and antibiotics need a sufficiently high and constant concentration to effectively reduce or kill bacteria. As the side effect profile increases with higher concentrations, it is particularly advantageous here to transport the active substance to the site of action by the shortest route, and to avoid systematic application in case of dermal or underling tissue infection.[63] To ensure a constant drug concentration, conventional formulations must be applied at regular intervals. Retardation of the dermal system is a promising approach for successful treatment and improved compliance.[64]

A film-forming iodophor preoperative skin preparation was developed by CareFusion. Prevail-FX® consists of Povidone-iodine, a complex of polyvinylpyrrolidone and triiodide, which is dissolved in isopropyl alcohol.[65] Investigations with several bacteria proved the antiseptic effect of the formulation. The film formed by the evaporation of the isopropanol remains on the skin long enough to reliably kill bacteria, unlike antiseptic solutions, which do not form a film.[65]

For the treatment of dermatological fungal infections, Mori et al. successfully developed a transdermal spray containing voriconazole. The drug was dissolved in a solution of ethanol and acetone. In each formulation, a combination of camphor and menthol was used as penetration enhancers and polyethylene glycol 400 was added. Several combinations of the film formers Eudragit® and Ethyl cellulose were compared to one another. A film-forming spray containing Eudragit® RLPO 10.05% and 5.02% Ethyl cellulose proved to be promising.[66]

Nicoli et al. studied the transdermal application of aminoglycosides. They were able to show that the permeation of amikacin in the form of a polymer film is similar to that of conventional gel and is suitable for dermal application. However, in order to make amikacin more readily available in deeper skin layers, procedures such as iontophoresis must be used.[67,68]

Another working group has looked at the dermal application of ciprofloxacin for the application to wounds. They created a non-water-soluble film by dissolving ciprofloxacin in an aqueous solution with polyvinylpyrrolidone and further excipients. This transparent film can protect a wound from external influences, and treats the infection at the same

time.[69] As there are already spray patches on the market that are used to close wounds, antibiotics such as ciprofloxacin could be integrated into them. The formed film would protect the wound from external influences and at the same time the infection would be treated specifically by the antibiotic depot contained in the film.

Yang et al. developed a film-forming solution for the treatment of MRSA infections in wounds. They developed a formulation of chitosan and polyvinyl alcohol with benzalkonium bromide. The film-forming solution was more suitable for the treatment of MRSA than the comparable aqueous solution with benzalkonium bromide.[70]

The topical application of local anaesthetics is another field of application for film-forming systems. On the other hand, a reduction in the concentration of the required drug, to avoid side effects and the prolonged effect at target site, is also the aim here.[71] For topical analgesia, Ranade et al. developed a film-forming spray with the non-steroidal anti-inflammatory drug ropivacaine. The formulations were made of the required drug and various concentrations of Eudragit EPO®, which were both dispersed in ethanol and isopropyl alcohol. The analgesic effect of this spray is comparable to that of a conventional lidocaine gel, but it can be applied more easily and less frequently, which increases patient compliance.[33]

In addition to conventional patches and other liquid or semi-solid preparations, the patch-no-patch system of Nicoli et al. could be established. They developed solutions of polyvinyl alcohol, Plastoid®, polyvinylpyrrolidone, sorbitol, glycerine, lidocaine HCL, and water, which were dried to a transparent film on silicone matrix. The selected skin area is moistened with water before application. Then, the film with silicone paper is placed on the skin area and the silicone paper is removed.[72] The transparent film adheres to the skin surface. The applied patch-no-patch film acts as a matrix-controlled patch which increases permeation, comparable to an aqueous lidocaine solution, without the undesirable side effects such as allergic reactions to the adhesive and cosmetic aspects.[73]

To compare different local anaesthetics, they also designed a patch-no-patch with benzocaine 3% and 5% instead of lidocaine, prepared with the same excipients. An in vivo testing in rats showed that the anaesthetic effect of benzocaine was increased compared to a semi-solid formulation, which was confirmed with ex vivo permeation testing. Another part of the study was the comparison of different penetration enhancers in ex vivo permeation. Transcutol®, propylene glycol, and isopropyl myristate were used

alone or in combination. The analgesic effect and transport of benzocaine could optimized by using a mixture of Transcutol® and propylene glycol.[74]

This patch-no-patch system was also developed of Femenía-Font et al. for sumatriptan, not for use as a dermal therapeutic system, but as a transdermal therapeutic system. The film was prepared by hydrating Polyvinyl alcohol in hot water and adding Plastoid® E35H and sorbitol. The sumatriptan succinate solution was incorporated to the mixture. Dependent on the film variant, Transcutol® or Polyethylene glycol 600 was added as penetration enhancer and ethanol as a volatile solvent. The film-forming solution was dried on silicone paper. The permeated amount of sumatriptan from the films was compared with the permeation of sumatriptan succinate solutions. It was found that the effect of penetration enhancers was the same for solution and film. In contrast to the solution, the release of sumatriptan into the skin was more controlled with the film, which is beneficial for the therapy.[75] The group also compared the permeation of Oxybutynin as a film-forming patch versus the conventional patch Oxytrol®. The patch-no-patch showed an increased permeation compared to the commercial patch. A lower drug loading and smaller application area can be employed.[76]

In developing a patch-no-patch system for the drug furosemide, Patel et al. compared the permeation at different ratios of ethyl cellulose and hydroxypropyl methyl cellulose, and tested the penetration enhancers propylene glycol, isopropyl myristate, and dimethyl sulfoxide. To produce the films, the drug and the polymers were dissolved in methanol and dichloromethane; di-n-butyl phthalate was added as a plasticiser and one of the three penetration enhancers. The solution was dried. The different films were tested for their moisture content and uptake, film-thickness, drug content, and in vitro permeation. The highest flux was determined at a ratio of ethyl cellulose and hydroxypropyl methyl cellulose of 8.5:1.5. Propylene glycol proved to be the best penetration enhancer.[31] Another working group around Amnuaikit also developed a transdermal film-forming patch-no-patch containing Propranolol hydrochloride successfully. As film-forming polymers, ethyl cellulose and polyvinyl alcohol were used in various ratios, always together with the plasticizer dibutyl phthalate. The optimal ratio was found to be twice the amount of ethyl cellulose compared to polyvinyl alcohol. The group also compared different penetration enhancers against each other. By using menthol and cineol as natural substances and propylene glycol as chemical enhancer. Cineole, or a mixture of cineole and propylene glycol, could increase the permeation of propranolol. Film thickness, drug content, and moisture uptake of the films were also investigated.[40]

Frederiksen et al. investigated film-forming solutions to achieve the sustained release of betamethasone 17-valerate into the stratum corneum. First, they compared films made of one polymer, which was dissolved or dispersed in ethanol/water solution together with the drug, without using further excipients. As film-forming polymers, hydroxypropyl cellulose, two polymethacrylate copolymers, and a polyacrylate copolymer were used. The release of betamethasone 17-valerat was highest when using hydroxypropyl cellulose as a polymer. The polymethacrylate copolymers and the polyacrylate copolymer have all showed a sustained release. Eudragit® RS performed best. Therefore, hydrophobic polymers are more suitable for reaching sustained release of hydrophobic drugs. On the other hand, the substantivity of the hydrophilic polymer hydroxypropyl cellulose is increased compared to the other polymers. This is an important aspect, especially for topical slow-release formulations. Another topic of the study was the influence of various plasticisers on the permeation of the cortisone derivative. For this purpose, triethyl citrate, tributyl citrate, or dibutyl sebacete were added in equal concentrations to the formulations with the hydroxypropyl cellulose polymer and Eudragit® RS. All plasticizers increased the permeation of the drug independently of the used polymer. The lipophilic plasticizer dibutyl sebacete increased the release the most, followed by tributyl citrate and triethyl citrate.[77]

2.4.2 Film-Forming-Gels

Gels are usually transparent and colourless, which makes them cosmetically appealing. Compared to solutions, gels offer the advantage of being easier and more accurate to apply as a semi-solid preparation. The advantage of using film-forming gel preparations instead of film-forming solutions is the easier application of the formulation due the semi-solid character. They are flexible, and mostly show high adherence to skin. Due to the added gelling agent, the polymer matrix is more viscous than comparable liquid preparations, which can be an advantage in the development of sustained release dosage forms. Compared to other semi-solid preparations, gels are particularly suitable for use on mucous membranes.[22]

In the field of wound healing, Kin et al. developed film-forming hydrogels with polyvinyl alcohol and polyvinylpyrrolidone as film-forming polymers, propylene glycol, ethanol, and water. The gel showed good adherence to skin and good protection of the wound from external influences. The gel can also serve as a base for the dermal application of drugs.[78]

Guo et al. developed an organic-inorganic hybrid gel, which combines the film properties of polyvinyl alcohol, the inhibition of crystallization by using polyvinylpyrrolidone, and the increase in mechanical properties with the use of γ-(glycidyloxypropyl)trimethoxysilane. To form the film-forming gel, Polyvinyl alcohol is dissolved in water and γ-(glycidyloxypropyl)trimethoxysilane is added. The used drug is dissolved in glycerol and polyvinyl pyrrolidone is added. This mixture is integrated into the polymer phase. The viscosity and adhesion to skin of the hybrid gel was investigated, as well as mechanical properties, water vapor permeability, and in vitro release. The gel showed a high adhesive residue on skin while it also exhibited high flexibility in the dry state. The formed film showed an optimal aesthetical appearance by virtue of being thin and transparent, and increased mechanical properties due the integration of γ-(glycidyloxypropyl)trimethoxysilane into the PVA matrix. The use of Polyvinylpyrrolidone and glycerol enhanced the drug permeation. The drug release of Ibuprofen and 5-Fluorouracil was sustained.[79] The permeation from the developed gel should be tested with other drugs in the future.

The working group around Li et al. successfully developed film-forming hydrogels for the transdermal application of tolterodine for the treatment of overactive bladder. The drug, together with triethanolamine was dissolved in water and ethanol and one or a combination of film-forming polymers such as poloxamer, hydroxypropyl cellulose, hydroxypropyl methyl cellulose, and methyl cellulose were added as gelling agent. The permeation and penetration experiments showed a rapid penetration of tolterodine into stratum corneum, in which the drug formed a depot. The bioavailability was high enough for transdermal action and was increased compared to oral bioavailability. The group was able to show that the transdermal application of active substances with film-forming gels is possible in general.[80]

2.4.3 Film-Forming Emulsions

Emulsions are liquid preparations and are usually made from an oil phase and a water phase. They are thermodynamically unstable and are therefore usually stabilised with the aid of an emulsifier. Both lipophilic and hydrophilic drugs can be incorporated into the two-phase or multi-phase system. In the case of film-forming emulsions, the used polymers act not only as film-forming agents but also as thermodynamic stabilisers of the emulsion, which may reduce the amount of emulsifier needed to stabilise the emulsion and therefore the risk of skin irritation.[81] Emulsions often show high compliance with the

patient, as they are pleasant to apply due to their usually lower viscosity and, in contrast to gels, still have a lipophilic phase without occluding properties.[82]

In this type of emulsion, the used polymer is dissolved or dispersed in the volatile solvent and forms the continuous phase. As the solvent evaporates, the viscosity of the polymer phase increases and stabilises the inner phase. The active ingredient is located in the droplets of the dispersed phase and diffuses through the polymer matrix into the skin. This in situ release enables a controlled sustained release of drugs, which is particularly interesting for the development of topical formulations for highly lipophilic drugs with a high affinity to stratum corneum. Due to the localisation of the active ingredient in the dispersed phase, supersaturation and crystallisation of the drug do not occur when the solvent evaporates. This is advantageous, as the thermodynamic properties of the emulsion would change with crystallisation and the emulsion stability could be impaired.

Lunter et al. achieved a sustained release of the active ingredient nonivamide by developing a film-forming emulsion. The compositions of the continuous phase were varied and the emulsions were tested for glass transition temperature, adhesion to skin, elongation, water resistance, and in vitro release. Nonivamide was dissolved in medium chain triglycerides and formed the dispersed phase of the emulsion. For the continuous phase, Eudragit® NE and RS were compared in different ratios, and hydroxypropyl methyl cellulose, polyvinylpyrrolidone, and polyvinyl alcohol were tested against one another as thickeners and polymer surfactants. Triethyl citrate and polysorbate 80 were used as plasticizers in various concentrations. The combination of water-soluble and water-insoluble polymers without using volatile solvent for formulation created the basis for further research in the field of film-forming emulsions.[83,84] The group also developed oil in oil emulsions with nonivamide in castor oil in the disperse phase and polydimethyl siloxanes as the continuous phase and silicone surfactant. The formulations were evaluated for drug content, phase volume, and viscosity of the silicone oil.[85] It has been shown that drug content and phase volume have an influence on the release rate and a sustained release of nonivamide over 12 h was achieved.[85]

Heck et al. used nonivamide in castor oil as a disperse phase. They loaded this oil mixture into silica particles and dispersed the particles in a continuous polymer phase of Eudragit® RS 30D and triethyl citrate by using different types of silica particles and preparation methods. The different formulations were tested for their homogeneity, storage stability, substantivity, and ex vivo permeation. Permeation experiments proved the sustained permeation of nonivamide passing the polymer matrix compared to a

commercial semi-solid formulation.[86] By loading the lipophilic disperse phase into the silica particles, it was possible to eliminate the need for an emulsifier in the manufacturing of the emulsion. Nevertheless, the formulation was physically stable. This process can be used and further developed in the future to reduce skin irritation.[87]

Schmidberger et al. developed thermogelling emulsions with nonivamide in medium-chain triglycerides as dispersed phase and varied the concentrations of methylcellulose as a film former, and the chain lengths of macrogol in the preparations. Other excipients were sodium citrate and water in the continuous phase, and ethanol as volatile solvent. The different formulations were tested for their rheological properties, droplet size, substantivity, and ex vivo penetration in experiments and compared to one another. The formulations showed increased substantivity compared to conventional semi-solid preparations. The high substantivity and the rheological properties were shown to be related. The substantivity increased with increasing methylcellulose concentration.[88] In order to quantify the substantivity, the working group was able to establish experimental set-ups to simulate the clothing-to-formulation and skin-to-formulation contact during ex vivo permeation experiments. They were able to show that the permeated amount of a conventional cream, the thermogelling emulsion, and the film-forming formulation after repeated contact to skin or clothing depends on their respective properties. With the help of this method, the impact of substantivity on permeation of different formulations can be reliably compared and optimised with regard to the concentrations and type of their ingredients.[89,90]

Padula et al. developed transdermal films and a microemulsion for the transdermal application of levothyroxine. For the microemulsion, isopropyl myristate and isobutanol were mixed and polysorbate, sorbianmonolaurate, and water were added. The levothyroxine was integrated into microemulsion and remained in the oily outer phase. The permeated amount of levothyroxine from the microemulsion was the same independent of the concentrations. In further steps, the microemulsions were loaded in the transdermal films which improved the skin retention of the levothyroxine which makes the concentration scalable and the use of this microemulsion as a sustained release formulation possible.[34]

2.5 Conclusions and Further Prospects

According to previous research findings, film-forming systems have proven to be suitable for use as a delivery form due to their high substantivity and cosmetic attractiveness.

Drugs can penetrate from the systems into the skin, which enables dermal and transdermal drug application. The formulations also serve as a depot, which enables a sustained release. While the use of film-forming sprays in the field of wound care is already established and well accepted by patients, film-forming systems are still the exception in the treatment of diseases.

In the field of transdermal application, there is, with the testosterone spray Axiron®, already one formulation on the market. For transdermal use, film-forming systems have not yet been able to establish themselves as a therapeutic option against other transdermal systems such as patches. For dermal application, only film-forming solutions for the treatment of topical infections, such as the terbinafine Lamisil Once® spray, have made it to the market; while studies on the treatment of chronic skin diseases were carried out, but no formulation is yet available for patients, neither for daily therapy in the form of cortisone preparations, nor as NSAIDs for acute therapy. The development of therapies for chronic inflammatory skin diseases and topical infections with film-forming formulations could be pursued more vigorously in the future.

Film-forming solutions that are sprayed directly onto the skin create the sensation of a second skin, unlike patch-no-patch systems. On the other hand, precise application and dosing are easier to implement by using patch-no-patch, which are also able to protect the treated skin area and show increased physicochemical stability. Film-forming emulsions also contain a lipophilic phase and can therefore also be used on dry skin areas. Due to their semi-solid character, gels have an advantage in that that they are easier and more precise to apply. All systems are well suited for personalised therapy. The semi-solid and liquid preparations can be produced in individual concentrations and the films can be printed individually.

Furthermore, there are comparatively few developments of film-forming formulations of drugs with hydrophilic character. A focus on the development of such formulations could provide access to further treatment fields.

The formulations can reach out more to the optimal condition of a second skin by optimising the concentration of the individual components and using new ingredients, thus increasing patient compliance. In this context, gels and emulsions can also become more important than the film-forming sprays that have been predominantly established to date, as they are easier to apply.

Another future field of research is the development of lipophilic film-forming systems with oil-soluble film-forming agents. These formulations could be used for chronic inflammatory skin diseases and, in addition to retarded release of the active ingredient

and high substantivity, have positive effects on skin properties. This would allow daily therapy with drugs such as cortisone derivatives to be combined with basic or maintenance therapy.

Author Contributions

Conceptualization, D.J.L.; methodology, D.J.L. and L.C.P.; investigation, L.C.P.; writing—original draft preparation, L.C.P.; writing—review and editing, D.J.L.; supervision, D.J.L.; All authors have read and agreed to the published version of the manuscript.

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3. Development of a film-forming oleogel with increased substantivity for the treatment of psoriasis

Larissa Carine Pünnel, Dominique Jasmin Lunter

Department of Pharmaceutical Technology, Eberhard Karls University, Auf der Morgenstelle 8, 72076 Tübingen

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3.1 Abstract

The aim of this work was the development of a film-forming formulation (FFF) for the topical treatment of psoriasis that shows an increased substantivity compared to conventional semi-solid dosage forms. The developed formulation is an oleogel. It is based on a combination of castor oil and medium chain triglycerides, and the oil-soluble film former MP-30 (Croda GmbH, Nettetal, Germany), a polyamide that upon mixing with a polar oil entraps the oil und thus substantially increases the viscosity of the formulation up to a semisolid state. Betamethasone dipropionate (BDP) and calcipotriole (CA) were used as active pharmaceutical ingredients (APIs). Oleogels of different compositions were evaluated regarding substantivity, rheological properties, ex-vivo penetration into the skin and ex-vivo permeation through the skin. Marketed products were used as controls. It was found that the amount of betamethasone dipropionate penetrating and permeating into and through the skin from the film-forming formulation is at an intermediate value compared to the marketed products. The substantivity of the developed formulation is described by an amount of 57.7 % formulation that remains on the skin surface and is thus significantly higher compared to the marketed products. In the film forming formulation, the proportion of API penetrating the skin remains the same when the skin repetitively brought in contact with a piece of textile during the penetration experiment. In contrast with the in-market formulations tested, this proportion was reduced by up to 97 %. As a result, the developed formulations can lead to an increased patient compliance.

Keywords: semi-solid dosage form, substantivity, ex-vivo skin penetration

3.2 Introduction

The human skin can be divided in two layers, the epidermis and the dermis. The dermis is located on top of the subcutis and is the lower layer of the skin.[1] It consists of connective tissue and is therefore particularly robust and gives the skin its flexibility. Blood vessels, hair follicles, sensory nerves and lymphatic channels are located in the dermis.[2] The epidermis on top can be divided into several layers. The proliferation of skin cells takes place in the stratum basale, which is located on top of the dermis, followed by the stratum spinosum and the stratum granulosum.[3,4] These two layers protect the skin from water loss.[5] The stratum corneum is the outermost layer of the epidermis, which is formed by dead corneocytes in a crystalline lamellar lipid matrix.[2] It forms an external barrier that protects the skin from external influences and water loss.[6] Usually, it takes 28 days for the cells produced in the stratum basale to reach the stratum corneum.[7] On their way they differentiate from keratinocytes to corneocytes.[8]

Psoriasis is a chronic inflammatory skin disease that mainly affects the skin, but can also affect other parts of the body such as joints and fingernails.[9] According to estimates, around 2 % of the population are affected, mainly by psoriasis vulgaris, which affects around 90 % of all patients.[9] The affected areas of the skin typical show scaly-shiny plaques on top of red skin and the patients suffer from itching and pain in the affected areas caused by inflammation and irritation of the dry skin.[10] Due to T-lymphocytes and other inflammation mediators, the dermis is inflamed, and proliferative activity of the epidermis is much higher.[10] The proliferation process of corneocytes just takes about 14 days. The poorly differentiated skin cells give the scaly appearance to the stratum corneum.[11] Furthermore, too many cells are formed in the stratum basale in an uncontrolled manner, which leads to a thickening of the entire epidermis.[12] This thick stratum corneum on top of the thickened epidermis of undifferentiated cells can no longer form an intact skin barrier, which leads to the risk of infections and increased water loss. The skin of psoriasis patients is thus very dry and requires the maintenance therapy with topical formulations with a high lipophilic content.[9] Furthermore, drug therapy is performed, where topical therapy is preferred over systemic therapy to circumvent to systemic side effects.[13] The combination of the two active ingredients betamethasone dipropionate and calcipotriole dispersed in a petroleum jelly base has been established as the gold standard in the topical therapy of psoriasis as they are synergistic.[14-16] As a corticosteroid, BDP binds to the intracellular corticosteroid receptors, which among other effects controls the transcription of proinflammatory

cytokines.[17] This results in anti-inflammatory, antiproliferative, and immunosuppressive effects, as well as inhibition of excessive cell proliferation in the stratum basale, thus reducing the silvery shimmering psoriasis plaques.[18,19] CA is a vitamin D3 analog that binds to the vitamin D receptor in the nucleus of epidermal keratinocytes and several types of inflammatory cells. It thus inhibits the expression of inflammatory mediators in the epidermis and dermis and reduces the redness of the affected skin areas.[20-23] Unfortunately, both active ingredients show low penetration and the petroleum jelly base does not show a high patient compliance.[24] The reasons for this are the sticky texture of the formulation and the fact that the formulation reaches unaffected areas through skin contact and leaves stains on clothing. Due to the reduced amount of preparation on the skin area, a reduced amount of the APIs is available for penetration and there is a risk that the therapeutic effect will not be achieved.

Substantivity is a parameter which indicates how well a formulation stays on the skin surface and is not worn off by other skin parts or clothing. [25,26] Formulations with high substantivity are able to form a drug reservoir on the skin and thus keep the required amount of APIs available for penetration. They may thus reduce the amount of formulation needed and/or the frequency of application of the formulation and thus improve patient compliance.[27]

The aim of the current study was the development of a semi-solid formulation for the treatment of psoriasis with BDP and CA, which contains a high proportion of lipophilic compounds and shows a higher substantivity compared to marketed formulations.[28] The marketed formulations Daivobet®, Enstilar®, Wynzora® and Xamiol® were used for comparison. Daivobet® is a petroleum-jelly based ointment.[12.21] Enstilar® consists out of the same ingrediencies as Daivobet® but is applied as a foam using diethyl ether. Wynzora® is a water based cream and Xamiol® is a castor oil based lipophilic gel.[29,30]

3.3 Materials and Methods

3.3.1 Materials

BDP, castor oil and medium chain triglycerides were obtained from Caesar & Loretz, GmbH, Hilden, Germany and CA from Cayman, Ann Arbor, USA. OleoCraft™ MP-30 was kindly donated by Croda, Snaith, Great Britain. Parafilm was acquired from Brand GmbH & Co. KG, Wertheim, Germany. Sodium chloride, disodium hydrogen phosphate and potassium dihydrogen phosphate, ammoniumformiate and acetic acid were of European Pharmacopoiea grade. The used methanol and dichloromethane were acquired from

Fischer scientific, Hampton, USA in LC-MS Grade and ultrapure water (Elga Maxima) was produced in house. The double-sided adhesive tape was produced by Tesa, Norderstedt, Germany and the used textile (B1302 enzyme-washed linen 140c) was produced by Hilco, Leinfelden-Echterdingen, Germany. The porcine ears were obtained right after death by a local butcher (Bio Metzgerei Griesshaber). The Institute of Pharmaceutical Technology is registered for the use of animal products with the Tuebingen District Office (registration number: DE 084161052 21, approval date: 22 December 2015).

3.3.2 Preparation of the Formulations

The test formulation is an oleogel. Castor oil, medium chain triglycerides and the film-former OleoCraft™ MP-30 were molten at 100 °C in an oil bath. The mixture was cooled down to 65 °C. About 20 % of this oleogel were used to form a pre-suspension of the APIs BDP in a concentration of 0.64 mg/g and CA in a concentration of 0.05 mg/g in a melamine bowl. The rest of the mixture was then added and stirred before the temperature of the mixture fell below 55 °C. The mixture was filled into the final containers while warm so that the gel structure could form therein. These formulations were used in ex vivo permeation and penetration testing as well as simultaneous ex vivo substantivity and penetration testing.

3.3.3 Design of experiments

A full factorial design of experiments (DOE) made by JMP® was used to develop an optimized prototype with the aim of maximizing substantivity. The three components OleoCraft™ MP-30, castor oil (CO) and medium-chain triglycerides (MCT) represented the input factors and were mixed in different proportions. Preliminary studies had shown that a concentration of the film former of less than 3 % leads to formulations that are too fluid and that formulations with a concentration of more than 7 % can no longer be spread. These concentrations were therefore defined as limits. Castor oil and medium-chain triglycerides were used in concentrations between 0-96 %. In total, 18 formulations were tested. The compositions are shown in table 1.

Avobenzone, as a chemical marker and surrogate API, was added at a concentration of 25 mg/g formulation to detect the amount of formulation that was worn off and the amount that remained on skin surface in substantivity tests. Each experiment was performed three times.

Table 1: Composition of the formulations for the design of experiments.

Formulation	MP-30 (m/m)	Castor oil (m/m)	Medium-chain-triglycerides (m/m)
1	0.070	0.465	0.465
2	0.070	0.930	0.000
3	0.030	0.009	0.961
4	0.070	0.465	0.465
5	0.050	0.576	0.374
6	0.030	0.961	0.009
7	0.070	0.000	0.930
8	0.030	0.961	0.009
9	0.070	0.930	0.000
10	0.030	0.465	0.505
11	0.050	0.593	0.357
12	0.070	0.000	0.930
13	0.030	0.961	0.009
14	0.030	0.485	0.485
15	0.050	0.000	0.950
16	0.050	0.000	0.950
17	0.070	0.930	0.000
18	0.030	0.009	0.961

3.3.4 Rheological characterization

To characterize the formulation rheologically, a plate-to-plate rheometer (Anton Paar MCR 501) with a 2.5 cm diameter plate was used. The formulations were equilibrated at 32 °C first and sheared at a shear rate of 5 1/s for 40 s. After a resting phase of 10 min the amplitude sweep measurement was conducted with a deformation between 0.01 and 1000 %. The storage modulus (G') and the loss modulus (G'') were measured with a frequency of 1H every 6 seconds for 100 points. The moduli were plotted against the amplitude, G' and G'' within the linear viscoelastic range (LVR), loss factor tan α (G''/G') and yield point (G'=G'') were determined to describe the gel character of the formulations.

3.3.5 Preparation of Porcine Ear Skin

Due to its similarity to human skin, pig ear skin was used for ex-vivo penetration and permeation experiments.[31] The porcine ears were obtained from the butchery Griesshaber (Moessingen, Germany). The ears were bathed in isotonic saline and dabbed off using soft tissue. The skin was separated from the cartilage and cleaned by using isotonic saline. The skin was cut into pieces with a scalpel, stretched onto aluminum foil wrapped Styrofoam blocks and fixed on the blocks with pins. The hair was shortened to 0.5 cm with a hair trimmer (QC5115/15 Philips Electronics, NL-Eindhoven, Netherlands) without harming the skin surface. A Dermatom (Dermatom GA 630 Acculan 3 TI Aesculap AG & Co. KG, Tuttlingen, Germany) was used to cut the skin to 1.0 mm thickness.[32] Circles of 25 mm and 30 mm diameter were punched out of the skin, wrapped in aluminum foil, and stored in a -28 °C freezer for a maximum of 6 months. On the day of the experiment the needed number of punches were thawed at room temperature.[33,34]

3.3.6. Substantivity testing: Texture analyzer

A texture analyzer was used to test the substantivity. About 20 mg formulation were applied to skin tempered to 32 °C, which was fixed to the lower die of a material tester (D=1,5 cm). A piece of textile was fixed to the upper die, which was pressed onto the formulation at a force of 10 N over 40 s.[25,35] The formulation was thoroughly removed off using a cotton swab. The textile and the cotton swab were extracted in 25 ml ethanol, extracted for 30 min in an ultrasonic bath and the avobenzone (DOE formulations) determined UV-spectrometrically using the Varioskan LUX (Thermo Scientific, Waltham, MA, United States) at a wavelength of 357 nm for the results, that are shown in 3.1. The calculated limit of detection (LOD) was 0.084 µg/ml and the limit of quantification (LOQ) was 0.25 µg/ml. The coefficient of determination r^2 for the calibration was 0.9898.

For the results that are shown in 3.4.3. In the case of API containing formulations, the skin and the textile were extracted in 10 ml ethanol, each for 30 min in an ultrasonic bath. The extracted BDP was determined using HPLC as described in 3.2.7.

3.3.7 Quantification via high performance liquid chromatography

For the quantification of BDP after substantivity testing with the texture analyser, HPLC (Shimadzu setup (CBM- 20 A, SIL- 20A, LC- 20AT, DGU- 20A SR) with a UV/Vis detector (SPD- 20 A, Shimadzu, Kyōto, Japan)) was used. With a C18 reverse-phase column (EC 125/4 NUCLEOSIL 100-5 C18, Macherey-Nagel GmbH & Co. KG, Dueren, Germany),

which was temperated at 35 °C an isocratic method was performed with acetonitrile and water 60:40 (V: V) and a flow rate of 1 ml/ min for 10 minutes. The BDP was detected after 4.7 minutes at a wavelength of 230 nm. The injection volume was 10 µl. The coefficient of determination r2 for the calibration was 0.9992. The calculated limit of detection (LOD) was 0.143 µg/ml and the calculated limit of quantification (LOQ) was 0.509 µg/ml.

3.3.8 Ex-vivo penetration and permeation through porcine postauricular skin

For the skin penetration tests Franz diffusion cells were used. The acceptor chamber was filled with PBS and equilibrated for 30 min at 32 °C. The acceptor medium was stirred with a speed of 500 rpm.[36]

Then, an amount of 50 mg of the test formulation was precisely applied on the skin. To protect the set-up from water loss, the donor compartment was covered with a piece of Parafilm and the sampling arm with a 1.5 ml Eppendorf tube.

After the incubation time the formulation was dabbed off the skin using two cotton swabs, which were subsequently extracted in methanol and water (1:1) in an ultrasonic bath for 30 min. The actual penetration area of the skin sample was punched out centrally to a size of 15 mm in diameter and frozen in liquid nitrogen. The remaining skin was extracted in 2 ml methanol and water (1:1) in an ultrasonic bath for 30 min, too. Figure 1 illustrates the procedure.

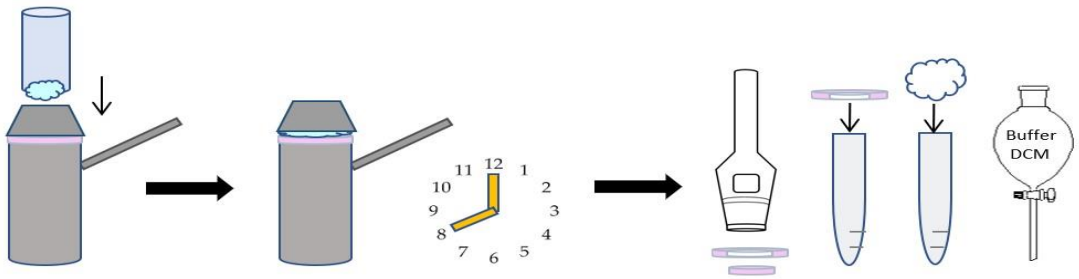

Figure. 1: Schematic representation of the penetration and permeation experiments in the Franz diffusion cell.

With a cryo-microtome (CM 1950, LEICA, Wetzler, Germany) the skin was sliced to divide the different skin layers. For the stratum corneum the first, incomplete cuts and the first complete cut with a thickness of 16 µm were collected. For the epidermis 10 sections with a thickness of 20 µm, and for the dermis 10 sections with a thickness of 80 µm.[37] All three sections were extracted in 1.5 ml methanol and water (1:1) in a 2 ml Eppendorf

tube and shaken for 30 min at 500 upm. The procedure had previously been validated to enable separation of the three skin layers.

To increase the concentration of the APIs in the samples of the acceptor medium and enable quantification by liquid chromatography mass spectrometry (LC-MS), they were extracted from the acceptor medium by 15 ml dichloromethane. After evaporation, the residue was dissolved in 1 ml methanol and water (1:1).

3.3.9 Simultaneous ex-vivo substantivity and penetration testing

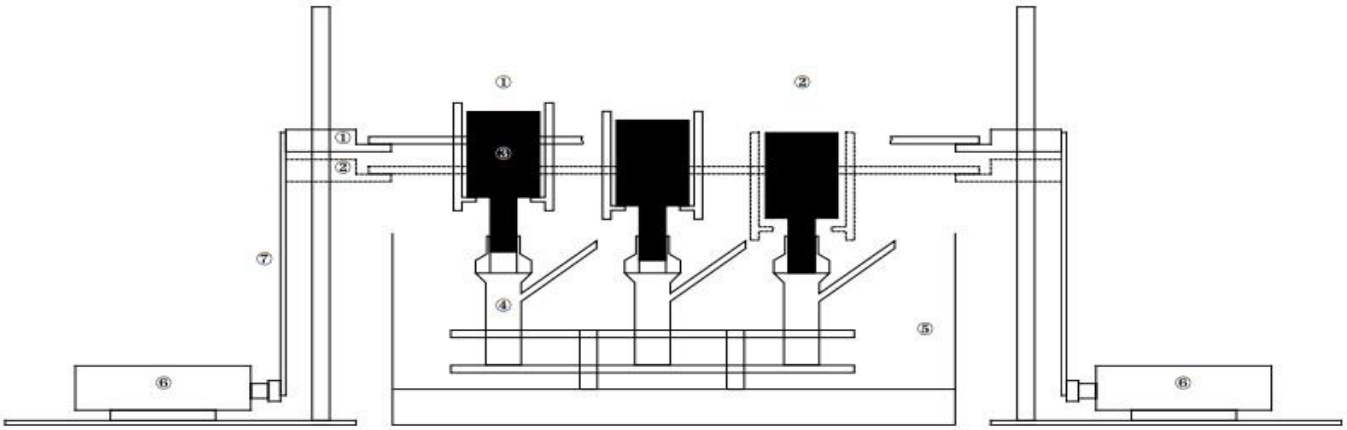

Figure 2: Drawing of the apparatus for ex-vivo substantivity and penetration testing. From [38]

The apparatus for ex-vivo substantivity and simultaneous penetration testing was built in the workshop of the Institute of Pharmacy at the University of Tübingen, and can be seen in figure 2. The lower end of the aluminum dies (3) with a weight of 132 g ± 1 g fit into the donor compartment of the Franz diffusion cells (4) and cover the entire surface of 1.77 cm2. A piece of textile was glued to each die using double-sided adhesive tape. The dies were placed into the appropriate holder. The holders were screwed onto the rail which was driven by two electric motors (6). These moved the rail up and down at a speed of 30 rpm. When moving down, the underside of the stamp touched the surface of the skin so that the skin and the piece of textile were in contact (2). The force with which the piece of textile was pressed against the skin surface was 0.65 N/cm2. The procedure was repeated for 5400 times (30 times per minute for 3 hours to simulate the contact of a piece of clothing on the skin in everyday life) 38. After 3h the dies were removed and the incubation of the skin was continued for another 9 hours.

3.3.10 Quantification via LC-MS

To determine the amount of BDP and CA, LC-MS (LC-MS 320, Agilent, Santa Clara, CA, USA) was used. The samples were chromatographically separated with a reverse phase

C8 column (EC 100/2 NUCLEODUR C8, Gravity 3 µm, Macherey- Nagel GmbH & Co. KG, Dueren, Germany) at a temperature of 40 °C. The flow rate of the mobile phase was set to 1 ml/min with the two flow agents water with ammonium formiate used in a concentration of 1 nM (A) and acetonitrile containing 1 nM formic acid (B). The separation started with a concentration of 30 % B and increased to a concentration of 70 % over 2.00 min. The concentration stayed constant over 5.00 min and afterwards the amount of B was decreased back to the 30 % over 1.00 min. The injection volume was 10 µl and the total chromatographic run time was 8.00 min. The retention time of CA was 4.46 min and that of BDP was 4.94 min. Both APIs were detected with a triple-quadrupole detector after electrospray ionization (ESI). A capillary voltage of 30000 V and a dwell time of 0.2 s were set and BDP was detected with an ESI positive mass of 411.3 m/z, as well as CA with an ESI positive mass of 395.4 m/z. Because of ionization effects the amount of BDP were calibrated out of the formulation themselves. The LOD for BDP in the Prototype was 0.316 ng/ml, regarding Daivobet® 0,495 ng/ml, Enstilar® 0.419 ng/ml, Wynzora® 0.372 ng/ml and Xamiol® 0.343 ng/ml. The LOQ were 1.76 ng/ml for the Prototype, 1.55 ng/ml for Daivobet®, 1.34 ng/ml for Enstilar®, 1.21 ng/ml for Wynzora® and 1.25 ng/ml for Xamiol®.

3.3.11. Statistical Data Analysis

Each experiment was performed three times. Statistical differences were determined by applying Analysis of variance (ANOVA) and followed by a Student-Newman-Keuls-Test at a significance level of 95 % (p=0.05) in Excel.

3.4 Results and discussion

3.4.1 Development of a film forming formulation

Previous film-forming formulations were developed with water-soluble film formers or non-water-soluble film formers, which were dispersed in water or organic solvents.[39,40] However, as the skin of psoriasis patients is very dry, the aim of the current study was to develop a lipophilic formulation with increased substantivity. The OleoCraft™ polyamide film formers LP-20, HP-31, MP-30 and MP-32 were selected for development. First, formulations were made with polar oils such as castor oil and medium-chain triglycerides, which resulted in uniform formulations with all four OleoCrafts™.

To determine the optimal concentration and combination of the different OleoCraft™ polymers and oils, formulations with concentrations of the individual film formers

between 1 and 20 % were prepared. The formulations with the two film formers LP-20 and HP-31 were pulling threads at all concentrations between 1 and 20 %, so that these were excluded for use in the formulation alone but were retained for possible combinations with the MP-30 and MP-32 film formers.

Since the film former MP-30 exhibited better substantivity at lower concentrations than the film former MP-32 and the formulations were less brittle it was selected for further development. The formulations with 1 %, 10 % and 20 % MP-30 and MP-32 were not spreadable. The formulations with 1 % were too fluid, those with 10 % and above were too solid and could no longer be spread. Therefore, formulations containing 1 to 10 % MP-30 or MP-32 were analyzed. The results show that concentrations between 3 and 7 % are acceptable for both film formers because the formulations can be applied and spread well.

Since the film former MP-30 exhibits a higher substantivity than MP-32 at the same concentration, further optimization of the formulations was performed using a DOE, as described in method 3.3.3. To prove the reproducibility and thus the validity of the experiment, two formulations were investigated in triplicates and four formulation were investigated in duplicates. Parameters for the design fit are r^2 = 0.93 and RMSE = 0,0997. CO, MCT and the combination MP30/CO/MCT had a significant influence on the substantivity. The substantivity for a desirability of 0.99 was set to 0.89. The polynomial equations for the three parameters MP-30, CO and MCT were:

$$y = 0.2135 + 1.221 * CO - 1.1257 * CO^2$$

$$y = 0.2404 + 1.171 * MCT - 1.266 * MCT^2$$

$$y = 0.3833 - 7.036 * MP30 + 103.3 * MP30^2$$

Figure 3 shows the result of the experiments from table 1. The result was that a combination of 7 % MP-30 dissolved in castor oil: medium-chain triglycerides 1:1 shows the highest substantivity. The concentration of the film-former has the highest impact substantivity. According to the evaluation of the DOE, the substantivity increases with increasing concentration of film-forming agents. Furthermore, it was found that the concentration of medium-chain triglycerides between 37.5 % and 48.5 % and a concentration of castor oil between 9 and 57 % would be optimal. It was therefore decided to use the formulation tested in the DOE consisting of 7 % MP-30 and equal parts of castor oil and medium-chain triglycerides for subsequent studies.

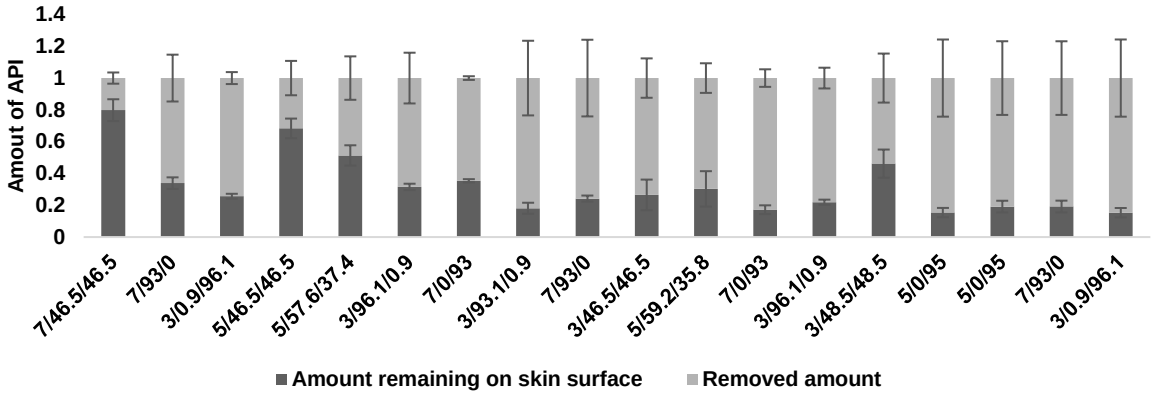

Figure 3: Results of the substantivity testing of the design of experiments with the concentration of MP-30/Castor Oil/ MCT (n=3, mean ± SD).

It was then tested whether this result could be improved by combining different film formers with a total concentration of 7% film former in equal parts of castor oil and MCT. The results are shown in Figure 4. The use of MP-32 alone and all combination with HP-31 and LP-20 could not achieve a comparable substantivity as the use of MP-30 alone. The combination of the film former MP-30 with LP-20 showed a comparable substantivity to the use of MP-30 alone while a combination with HP-31 decreased substantivity. Since no formulation could achieve better substantivity than the formulation containing 7% MP-30 in equal parts of castor oil and medium-chain triglycerides, this one was selected as the most promising formulation and subjected to further characterization in comparison to the marketed products.

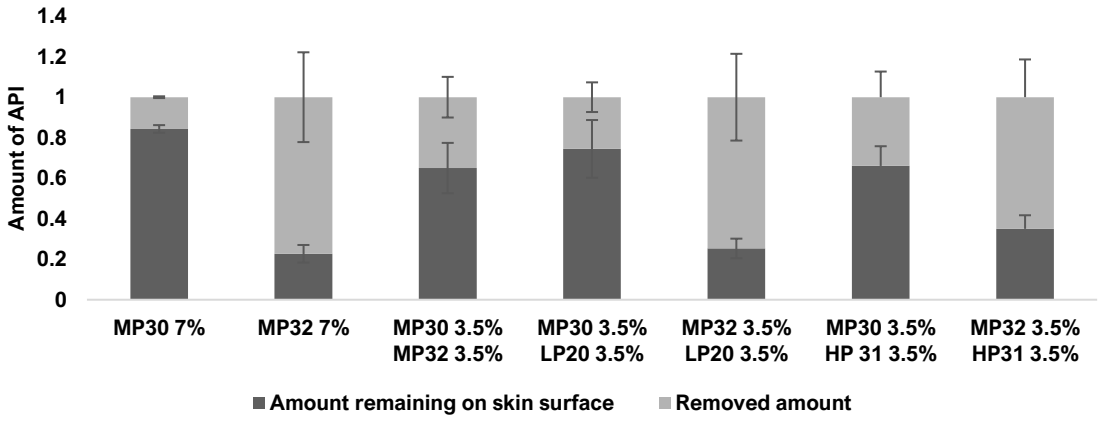

Figure 4: Results of the substantivity testing of the comparison of different combination of OleoCraft™ polymers (n=3, mean ± SD).

3.4.2 Rheological characterization

The developed formulation and the marketed products were characterized rheologically, shown in figure 5. The dynamic module and the loss module were determined for each formulation and the loss factor was calculated from them. Furthermore, the yield point was determined.

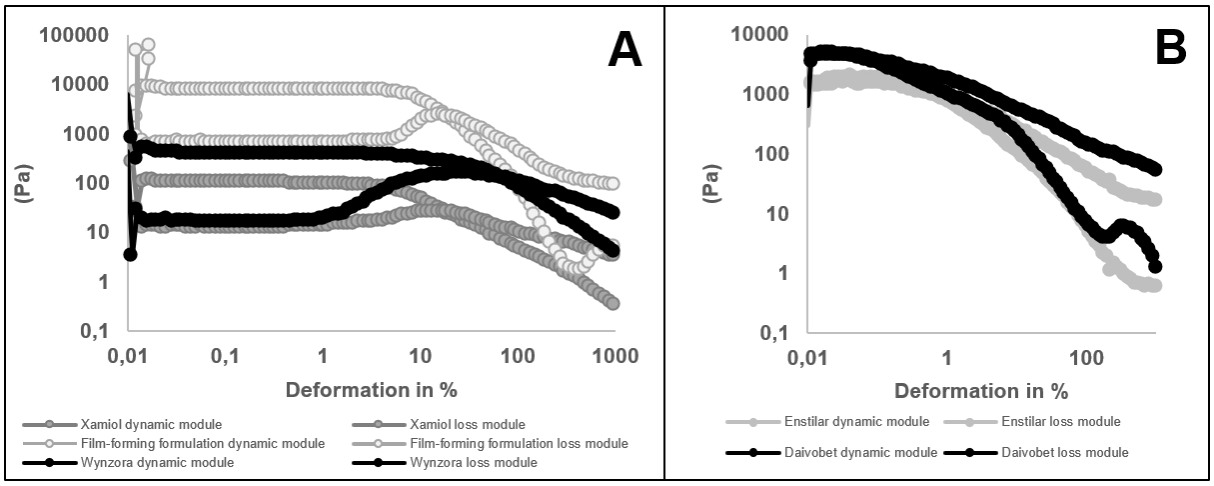

Figure 5 a,b: Results of the rheological characterization of the formulations Xamiol®, Wynzora® and the film-forming formulation (A) and the marketed products Daivobet® and Enstilar® (n=3, mean ± SD).

All formulations can be characterized as semi-solid, as they initially resume their shape elastically, but are made to flow by increased shear forces and can thus be distributed easily on a surface, e.g. the skin. The two petroleum-jelly based formulations Enstilar® and Daivobet® start to flow at a low deformation. The yield point of Daivobet® is at 0.01 % deformation and the yield point of Enstilar® at 0.02 % deformation. Both, the storage modulus G' and the loss modulus G'' are higher in Daivobet®: G' of 1600 Pa and G'' of 1500 Pa for Enstilar® compared to G' of 5000 Pa and G'' of 4450 Pa for Daivobet®. The yield points of the film-forming formulation, Wynzora® and Xamiol® are higher compared to the petroleum-jelly based formulations. The yield point of the three formulations could be determined at a deformation of 21.4 % for the film-forming formulation, 21.1 % for Xamiol® and 86.0 % for Wynzora®. The film-forming formulation shows an elastic behavior with G' of 7950 Pa and G'' 7130 Pa, while Wynzora® gives lower muduli with G' of 430 Pa and G'' of 17 Pa, followed by Xamiol® with G' of 104 Pa and G'' of 13 Pa.

The film-forming formulation, Wynzora® and Xamiol® show loss factors of 0.09 for the film-forming formulation, 0.04 for Wynzora® and a loss factor of 0.14 for Xamiol® and thus resemble rather viscoelastic bodies while Enstilar® and Daivobet® resemble rather ideal-

viscous bodies. This means that the two ointments deform plastically immediately when force is applied and can be spread over the skin surface easily.

3.4.3. Substantivity

The film-forming formulation for further investigation was selected based on its superior substantivity compared to the other formulations of the DoE. Substantivity was aimed to be higher than that of the marketed formulations, while the formulation should still be easily spreadable. The substantivity of the developed formulation was thus compared to that of the marketed formulations. The results are given in figure 6.

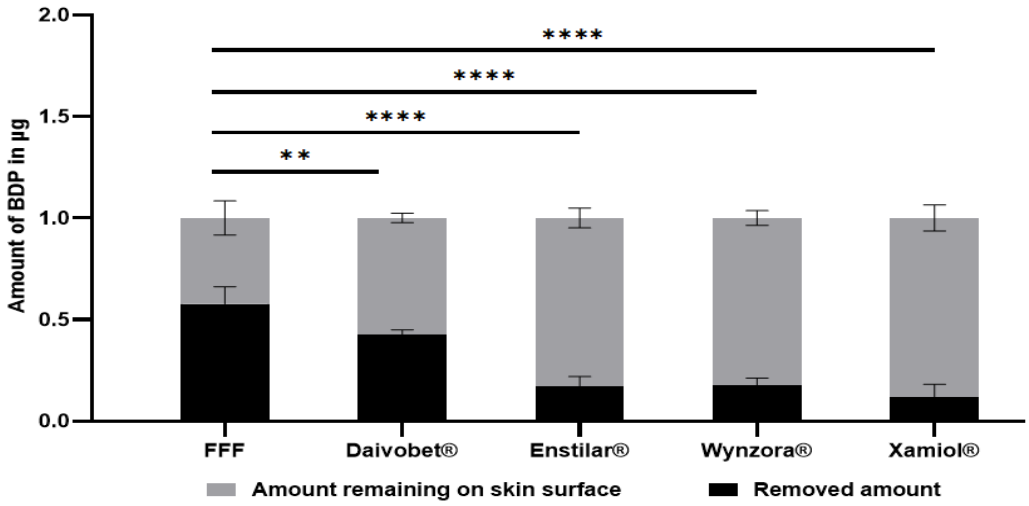

*Figure 6: Results of the substantivity testing n = 3; mean ± SD. ** p< 0.01, **** p< 0.0001*

The film forming formulation shows the highest substantivity (57.7 %), followed by the in-market product Daivobet® with 42.6 %. The amount of formulation that was removed from the skin surface was higher regarding the in-market products Wynzora® with 82.3 %, Enstilar® with 82.9 % and afterwards Xamiol® with an amount of 88.3 %. The film-forming formulation differs statistically significantly from all marketed formulations.

3.4.4. Ex-vivo penetration and permeation through to auricular porcine skin

The film-forming formulation and the marketed formulations were compared in a 24 h penetration experiment. The aim was to show that the film forming formulation is comparable to the marketed formulations. All data show the results of the penetrated and permeated amount of BDP that was used in a concentration of 0.64 mg/g in the formulation. The quantities of CA which penetrated and permeated the skin could not be reliably detected because CA is degraded on the skin surface already and the quantities were below the LOQ. The amount of BDP that penetrates the skin to inhibit excessive

cell proliferation could be detected and quantified. The barrier for this is the stratum corneum. Compared to the epidermis, the stratum corneum is very lipophilic, which creates the risk that the lipophilic API remains in the stratum corneum and does not penetrate into the more hydrophilic epidermis. Due to the risk of systemic side effects and to avoid systemic absorption, the drug should only penetrate the underlying dermis in small quantities. It was found that BDP penetrates out of all five formulations into and through the skin at all investigated incubation times. The largest amount of API which penetrates the skin was measured for Enstilar®. The difference is statistically significant. The results are shown in figure 7 and in table 2. The penetrated amount of BDP out of the film-forming formulation and the marketed formulations Daivobet®, Xamiol® and Wynzora® are not statistically significantly different. It can thus be concluded that the amount of API delivered to the skin from the film-forming formulation is within a suitable range for therapy.

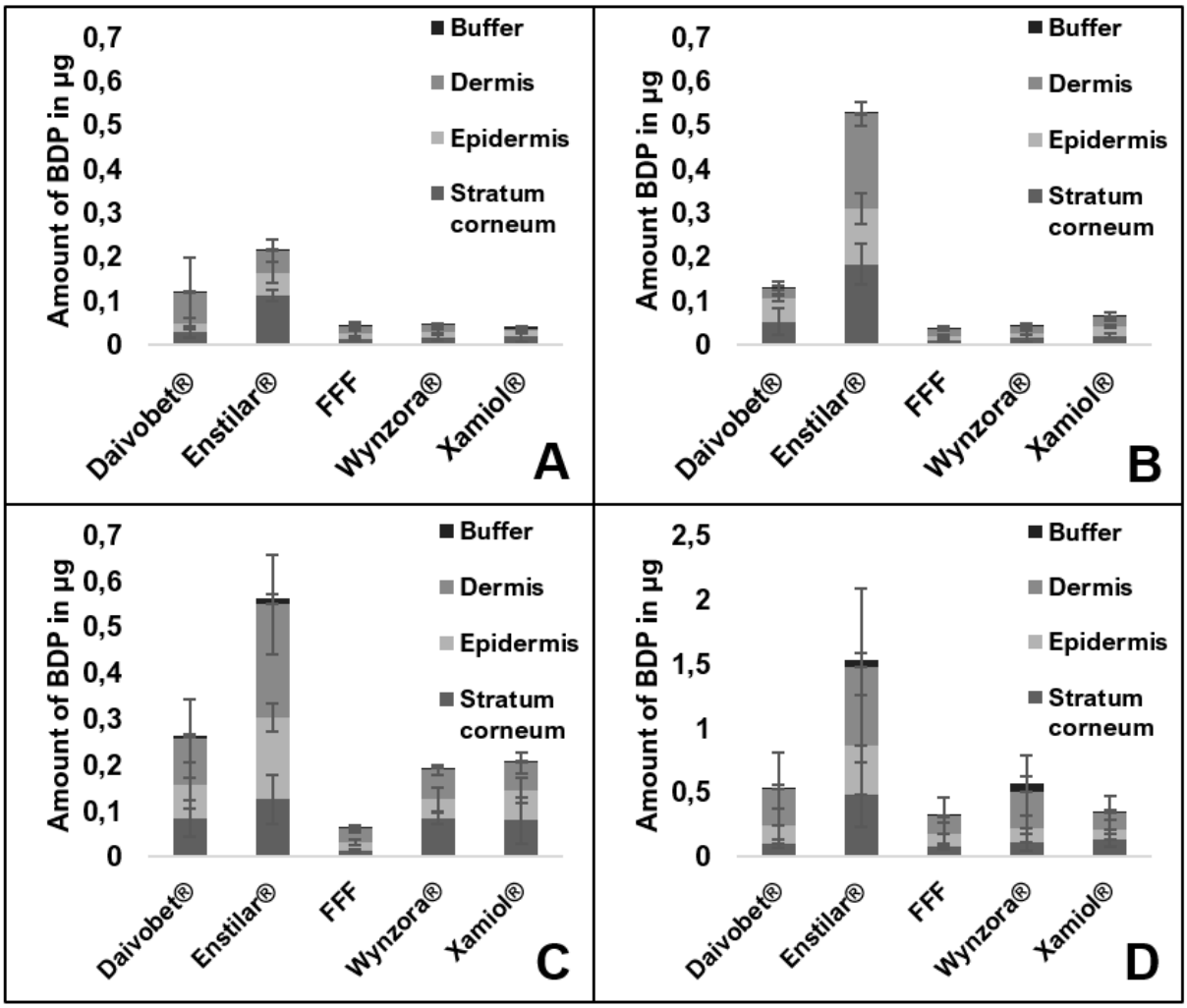

Figure 7 a-d: Penetrated amount of BDP into the different skin layers after 3 h Incubation (A), 6 h incublation (B), 12 h incubation (C) and 24 h incubation (D) (n=3, mean ± SD)

Table 2: Penetrated amount of BDP into the skin.

	Penetrated amount of BDP in ng after incubation time			
Incubation time	**3**	**6**	**12**	**24**
Enstilar®	112.1 ± 36.1	469.7 ± 108.4	619.4 ± 114.1	1531.9 ± 302.5
Daivobet®	29.3 ± 70.2	129.9 ± 53.9	263.7 ± 154.3	542.9 ± 190.6
Wynzora®	17.6 ± 12.9	43.2 ± 13.5	194.9 ± 32.6	567.2 ± 181.5
Xamiol®	21.3 ± 15.0	65.2 ± 15.5	207.6 ± 7.88	350.7 ± 63.1
Film forming formulation	12.8 ± 13.1	37.6 ± 10.9	62.7 ± 14.3	321.9 ± 68.5

The kinetics of penetration were determined at four points over a period of 24 hours. It was found that the amount of BDP penetrating the skin increases linearly regarding the marketed products and exponentially regarding the film-forming formulation as shown in figure 8.

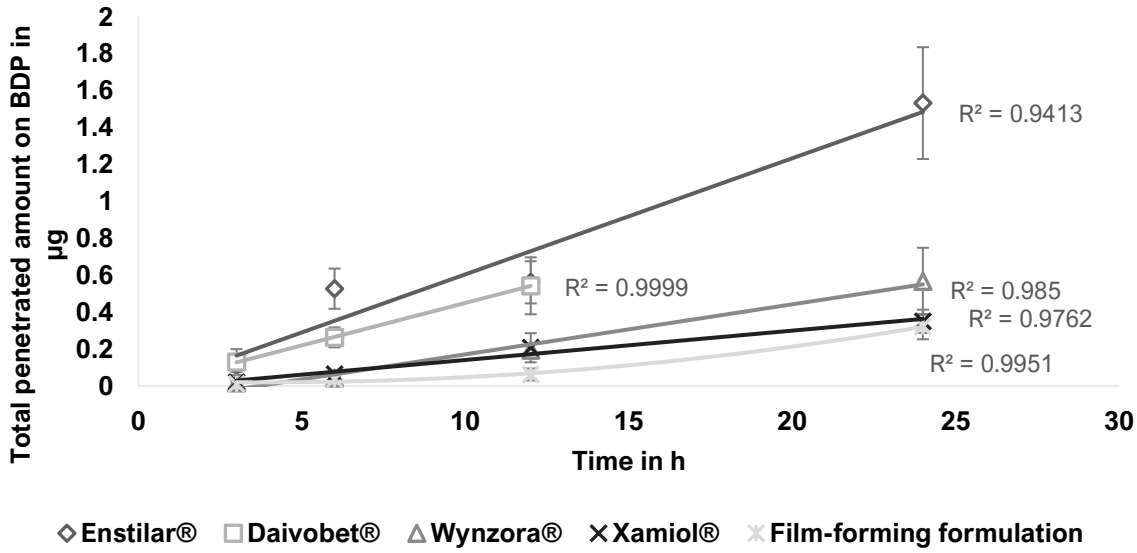

Figure 8: Total penetrated amount of BDP in µg over 24 h (n=3, mean ± SD).

The permeated amount is the amount of drug that passes from the applied formulation through the skin into the acceptor medium and is a surogate of the systemically absorbed dose. Due to systemic side effects, the drug should not enter the bloodstream.

Table 3 shows that the amount of BDP that permeates through the skin from all formulations is very small (2 – 64 ng). Since the log P value of betamethasone dipropioate is approx. 3.6, it was to be expected that this lipophilic drug would permeate poorly into a hydrophilic system. The standard deviation of the permeation values are rather high. The reasons for this are the fact that pig skin is a natural specimen that exhibits fluctuations although all procedures were maximally standardized and that the permeated amounts of BDP after the 3 h experiment could be detected but not reliable quantificated. Therefore, no significant difference was found between the formulations

regarding the permeation experiments. The body's own cortisol production is around 9.5 mg per day.[41] BDP is around 25 times more potent than cortisol, which would correspond to a daily amount of 237.5 µg BDP.[42] Even with the large standard deviations, the highest detected quantity of BDP in the buffer corresponds to only 0.026 % of the quantity produced daily. In patients with mild to moderate psoriasis, up to 5 % of the body surface is affected. In patients with the severe form, which can still be treated without systemic antibodies, it is a maximum of 20 %. Are 20 % of 2 m2 body surface area treated with the formulation, the dose corresponds to around 62 ug for a single application. It can therefore be stated that it is very unlikely that there is a systemic effect to be expected when one of the five formulations is used, even if a larger skin surface is treated.

Table 3: Permeated amount of BDP through the skin (n=3, mean ± SD):

	Permeated amount of BDP in ng after incubation time			
Incubation time	**3**	**6**	**12**	**24**
Enstilar®	1.43 ± 1.67	1.14 ± 0.10	12.43 ± 9.79	54.50 ± 48.09
Daivobet®	2.45 ± 1.13	2.03 ± 0.52	5.87 ± 4.07	17.49 ± 14.33
Wynzora®	0.66 ± 0.34	1.65 ± 1.29	5.61 ± 2.76	62.42 ± 0.73
Xamiol®	0.36 ± 0.62	1.44 ± 0.62	2.74 ± 1.42	10.30 ± 25.34
Film forming formulation	1.76 ± 0.79	1.36 ± 1.86	1.38 ± 0.57	1.95 ± 2.81

3.4.5. Ex-vivo penetration and permeation through to auricular porcine skin with substantivity testing

As the aim of the development is a formulation with increased substantivity, the penetration tests were repeated under more realistic conditions for the incubation time of 12 hours. As described in method 3.3.8, a piece of textile touched the skin surface 30 times per minute for 3 hours to simulate the contact of a piece of clothing on the skin in everyday life. The skin was then incubated for a further 9 hours. As can be seen in figure 9 a and table 4, with regard to the marketed formulations, between 41 % (Daivobet®) and 79 % (Xamiol®) of formulation are removed from the skin. The least amount of formulation was removed from the film-forming formulation with only 19 %.

With the reduced amount of formulation on the skin surface, the amount of BDP which penetrated the skin after 12 hours was reduced regarding all formulations. Figure 9 b shows the penetrated amount of BDP into the different skin layers of this experiment. Figure 9 c compares the results of the penetration experiment after 12 h from experiment 3.4.4 with those of this experiment. In the results from 3.4.4 it is described that most BDP

from the Enstilar® formulation penetrates the skin. This amount was significantly higher than out of the other tested formulations. There was no significant difference among each other regarding the other formulations.

However, this more realistic test setup shows that the amount of BDP that penetrates the skin out of the film-forming formulation is lower than the amount that penetrates out of Daivobet® and Enstilar® but higher than the amount that penetrates out of Xamiol® and Wynzora®. Table 4 shows the percentage by which the amount of BDP penetrated the skin was reduced due to repeated textile contact compared to the experiment without textile contact (section 3.4.4). Out of the film forming formulation 53 % of the applied BDP penetrated the skin. This percentage is higher than that of all marketed formulations. The penetrated amount from the film forming formulation was reduced by 47 % due to the repeated textile contact. The amount penetrated from the marketed formulations is reduced by 77 to 97 %. This again confirms the higher substantivity of the film forming formulation. The total amount penetrated after repeated textile contact is 33 ng from the film forming formulation and 6 to 60 ng from the marketed products. As the marketed products have proven their efficacy in in vivo studies where repeated textile contact must be assumed, the BDP amounts found in the skin after textile contact may be assumed to be suitable for therapy and thus, the film-forming formulation also shows a penetrated amount that falls within a range suitable for therapy. An important advantage of the film forming formulation is the fact that the reduced amount of formulation that is removed by the textile in turn reduces the consumption of drug product as well as the risk of the formulation polluting the patients environment and surrounding people with the API. This represents an important improvement with respect to drug safety.

Table 4: Results of the substantivity testing during penetration [%].

	Daivobet®	**Enstilar®**	**FFF**	**Wynzora®**	**Xamiol®**
Skin surface	0.59	0.28	0.81	0.08	0.21
Textile	0.41	0.72	0.19	0.92	0.79
Penetrated amount 12h in ng	263.69	561.75	61.92	194.98	207.56
Penetrated amount 12h in ng Substantivity testing	60.61	55.81	32.68	5.57	11.86
Amount BDP that still penetrated the skin	0.23	0.10	0.53	0.03	0.06
Reduced Amount	0.77	0.90	0.47	0.97	0.94

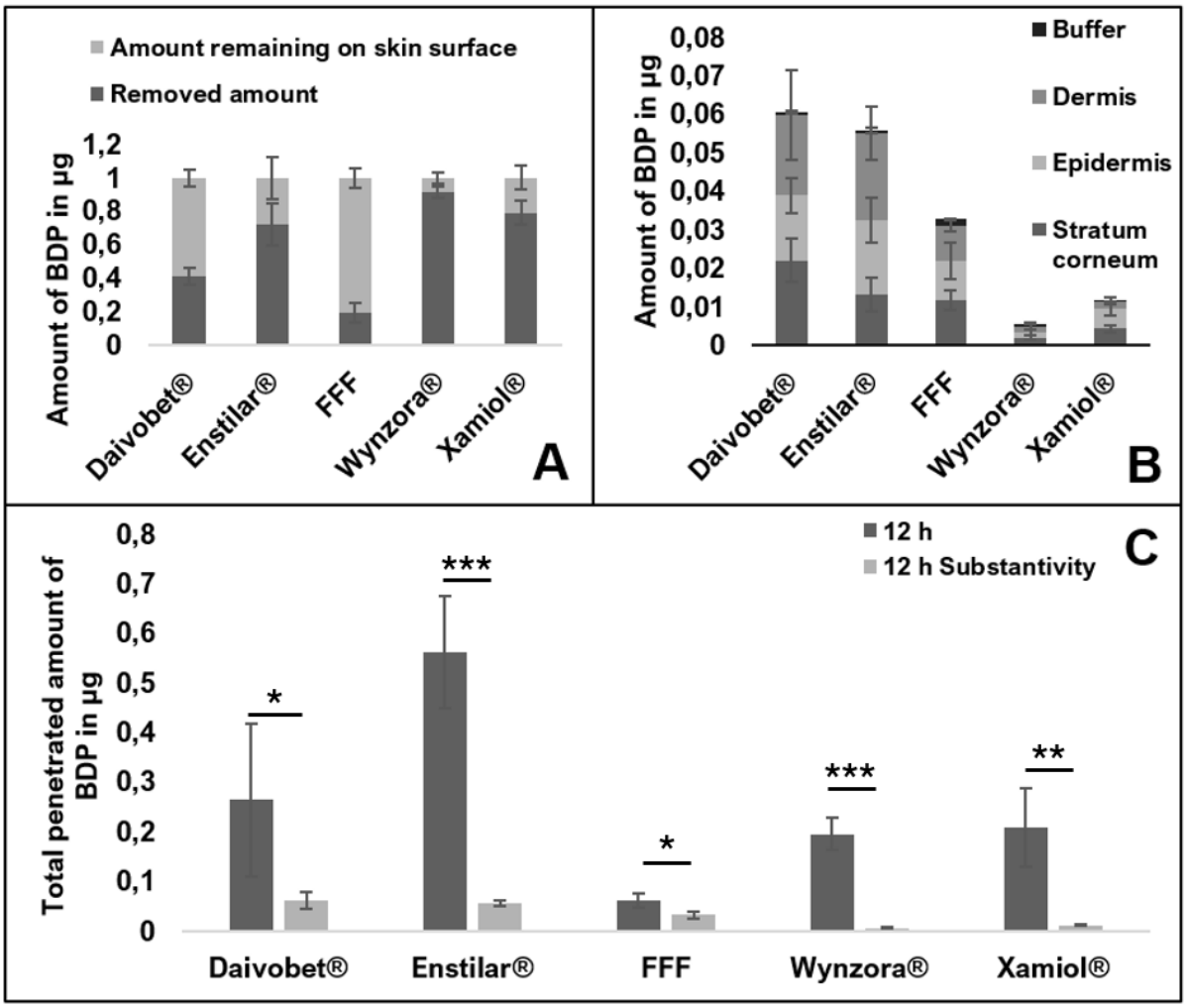

*Figure 9 a,b,c:: Substantivity of the formulations after 3 h testing (A) and results of penetration testing after 12 h incubation (B) and the penetrated amount of BDP out of the formulations in and through the skin after 12 h incubation and the amount after 12 h with simultaneous substantivity testing (C) (n=3, mean ± SD). * p< 0.05, **p< 0.01, *** p< 0.001*

3.5. Conclusion

The aim of this study was the development of a formulation with the BDP and CA for the treatment of psoriasis with increased substantivity compared to the marketed products Enstilar®, Daivobet®, Xamiol® and Wynzora®, with comparable penetration of the active ingredient BDP into the skin. Since the skin of psoriasis patients is dry, a vehicle with a high lipophilic content was developed. Castor oil and medium-chain-triglycerides were used in equal proportions. To form a semi-solid formulation with a high substantivity, the lipophilic polyamide film-former Oleocraft™ MP-30 was used. The developed formulation is a semi-solid formulation showing a remarkable substantivity which was found to be

significantly enhanced compared to the marketed products. The penetration studies verified a comparable amount of BDP that penetrates the skin and comparable penetration kinetics. The permeation examinations verify that the amount of BDP permeated through the skin is not a risk for systemic side effects. The combination of the substantivity testing together with the examination of penetration verified that the increased substantivity of the developed formulation results in the penetration of BDP from the film-forming formulation being reduced to a significantly lower degree as from the marketed formulations. To conclude, the developed film-forming formulation is a promising formulation for the treatment of psoriasis. It is water free, easy to modify and thus could open a concept for topical treatment with lipophilic drugs that is superior to the frequently used petroleum-jelly as the increased substantivity can reduce the frequency of application and therefore increase patient compliance.

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Conflict of interest

The authors declare not conflict of interests.

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4. Development of 3D printed microneedles of varied needle geometries and lengths, designed to improve the dermal delivery of topically applied psoriasis treatments.

Larissa Carine Pünnel [1], Maria Palmtag [2], Dominique Jasmin Lunter [1], Jillian L Perry [3]

1. Department of Pharmaceutical Technology, Eberhard Karls University, Auf der Morgenstelle 8, 72076 Tübingen, Germany

2. Lineberger Comprehensive Cancer Center, University of North Carolina at Chapel Hill, Chapel Hill, NC, 27599 (USA)

3. Eshelman School of Pharmacy, Department of Pharmacoengineering and Molecular Pharmacology, Center for Nano and Drug Delivery, University of North Carolina at Chapel Hill, Chapel Hill, NC 27599 (USA)

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4.1 Abstract

The aim of this study was to investigate the impact of using microneedle patches in addition to topical therapy for the treatment of psoriasis. Using continuous liquid interface production (CLIP) 3D printing we manufactured round microneedle array patches (MAPs) with a diameter of 14 mm. Needle geometries were varied from square pyramidal, conical, and obelisk, with varied needle lengths of 400 µm, 600 µm, 800 µm, or 1000 µm. MAPs were characterized for force to fracture, skin penetration, skin damage, as well as their ability to deliver a novel oleogel-based corticosteroid (betamethasone dipropionate (BDP) formulation into ex-vivo porcine skin. We found that the obelisk shaped MAPs are more durable compared to the conical and square pyramidal-shaped MAPs. When the obelisk shaped MAPs were used in combination with the oleogel-based BDP formulation, the amount of BDP penetrating the skin was significantly increased with greater needle lengths.

4.2 Introduction

Psoriasis affects approximately 2 % of the world's population[1], with approximately 90 % of cases corresponding to psoriasis vulgaris, or more commonly known as plaque psoriasis.[2,3] Plaque psoriasis typically presents as large oval-circular patches of dry, thickened, raised, inflamed skin, covered with a buildup of dead skin cells of the stratum corneum termed scale. Psoriasis is also characterized by a diminished skin barrier, resulting in increased water loss, promoting dry skin and an increased risk of infection. Topical treatments using ointments or creams are the standard of care for mild to moderate plaque psoriasis. An established gold standard for topical therapy of psoriasis is a combination of betamethasone dipropionate (BDP), a corticosteroid, and calcipotriole (CA), a vitamin D3 analog.[4] Treatment with this combination reduces the local inflammation and the scaly and silvery appearance of the skin, as well as decreases the skin thickness and improves the skin barrier. Topical therapies containing these active ingredients are typically petroleum-jelly based, which leaves a greasy feeling on the skin surface and is easily wiped from the skin by contact with clothing, necessitating multiple applications per day, which results in poor patient compliance when it comes to regular use.[5] We have recently reported on a novel oleogel-based film forming formulation (FFF), that resulted in enhanced BDP delivery into the skin in a substantivity test.[6] While improving substantivity of topical treatments is critical, researchers continue to focus on methods to improve dermal penetration, as the epidermis of psoriasis patients can be up to five times thicker than in patients without psoriasis, making it challenging to topically deliver APIs deep into the thickened skin.[7]

One approach to improve dermal delivery is through the utilization of self-applicable microneedle array patches (MAPs), which consist of micron-sized needles, arrayed over a base plate.[8] The application of microneedles onto skin, physically pierce the stratum corneum (the skin's outermost layer), allowing for direct cargo delivery into the dermis.[9-11] The typical lengths of the microneedle projections range from 500 µm to 1000 µm, and have demonstrated skin penetration of approximately 250 - 300 µm.[12-14] This depth of penetration avoids penetration of the dermal layer, where blood vessels and nerve fibers are located, and therefore MAP applications are often referred to as pain free.[10,15] Based on their ability to effectively puncture the stratum corneum in a pain free manner, utilization of microneedles for the treatment of psoriasis is gaining interest.[5,16] Furthermore, a pilot clinical study conducted in 2016 reported improved efficacy for topical psoriasis treatments if they were followed by application of 650 µm

tall hyaluronic acid (dissolvable) MAPs.[17] MAPs of similar designs to those used in this clinical study, have demonstrated skin penetration of approximately 300 μm, and thus the ability to penetrate through healthy human stratum corneum and embed with the epidermis. Since the skin of psoriasis patients is thicker, we hypothesized that the efficacy of microneedles manufactured for use on psoriasis could be improved by manufacturing optimized needles for deeper skin puncture to account for penetrating into thickened skin. While it is accepted throughout the literature that that longer microneedles can penetrate deeper into the skin,[12,18,20] literature reports have demonstrated that the interplay between needle composition, needle geometry, and length, influence needle mechanical strength, depth of skin puncture, and ultimately the amount of cargo delivered into the skin.[18-20] While square pyramidal needles are used predominantly throughout the literature, reports have demonstrated that conical or obelisk-shaped microneedles do have advantages over this classic design.[19,21] Studies have shown that conical shaped microneedles can penetrate deeper into the skin, potentially due to their sharper tip angle, and enhanced mechanical stability.[22,23] Obelisk microneedles have also demonstrated higher mechanical stability compared to other designs[19], as well as larger surface area per needle, allowing for more drug to be loaded on the needles.[24] In this study to test our hypothesis, we systematically evaluated the impact of needle length and geometry on needle mechanical strength and depth of skin puncture, to ultimately down select a single needle geometry to demonstrate the impact of needle length on cargo delivery into the skin.

While molded dissolving MAPs are heavily used throughout the literature, adjusting needle designs is a laborious process in which first masters need to be manufactured, followed by the production of molds. Lastly, the manufacturing of the molded MAPs is a multi-step process often taking multiple days. Herein, to systematically investigate the effect of needle geometry (square pyramidal, conical, and obelisk) and length (400-1000 μm) on needle stability (force to fracture), depth of skin puncture, skin damage (using trans epidermal water loss readings), and dermal delivery of BDP from our novel FFF, we have utilized a rapid 3D manufacturing process termed continuous liquid interface production (CLIP), that has been utilized previously to rapidly manufacture polymeric microneedles.[21, 25-28] CLIP technology is based on digital light synthesis, in which solid parts are produced from a liquid resin (Figure 1). The speed of CLIP production is achieved by using an oxygen permeable window, which results in a thin layer of oxygen rich resin (termed ‘the dead zone’) above the window. The oxygen in the resin inhibits free radical polymerization, and therefore the ‘dead zone’ results in a layer of uncured

resin between the window and the printed part, allowing for continuous 3D printing, and unparalleled print speeds.[29]

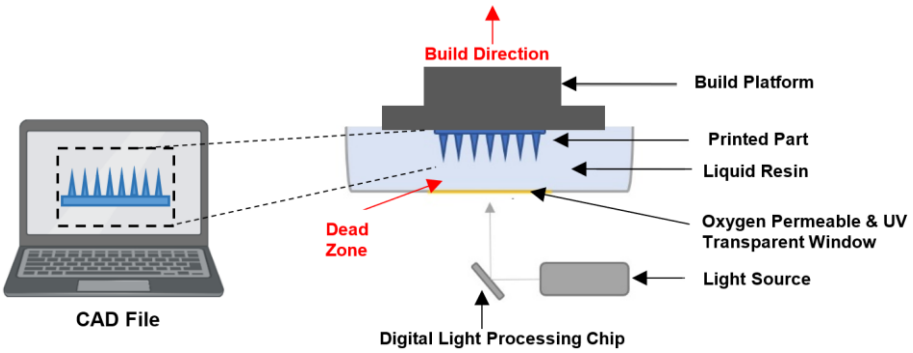

Figure 1. Schematic of the continuous liquid interface production technology. 3D parts are first designed using a computer aided design (CAD) program and then uploaded onto the printer software. Light projections based on 2D sectioning of the 3D design are then flashed under the window, curing the part to the build platform. The light continues to shine as the build platform raises in the z-direction to continuously build the 3D part.

4.3 Methods and Materials

4.3.1 Materials

Betamethasone dipropionate, castor oil and medium chain triglycerides were obtained from Caesar & Loretz, GmbH (Hilden, Germany). Calcipotriole was obtained from Cayman Chemical (Ann Arbor, United States). The OleoCraft MP-30 was kindly donated by Croda (Snaith, United Kingdom). Polyethylene Gylcol was acquired from TCI and the TPO and BLS from Aldrich (St. Louis, United States). Parafilm M® was acquired from Brand GmbH & Co. KG (Wertheim, Germany). Sodium chloride, disodium hydrogen phosphate and potassium dihydrogen phosphate, ammoniumformiate and acetic acid were of European Pharmacopoiea grade. The used isopropanol, methanol and dichloromethane were acquired from Fischer scientific in LC-MS Grade and ultra-pure water was used. The porcine ears were purchased right after death by a local butcher (Bio Metzgerei Griesshaber, Moessingen-Oeschingen, Germany). The Institute of Pharmaceutical Technology is registered for the use of animal products with the Tuebingen District Office (registration number: DE 084161052 21, approval date: 22 December 2015). Polyetheylene glycol dimethacrylate (PEG350DMA) was purchased from TCI America (Portland, USA) Diphenyl-(2,4,6-trimethylbenzoyl) phosphine oxide

(TPO), and 2-tert-Butyl-6-(5-chloro-2H-benzotriazol-2-yl)-4-methylphenol (BLS) were purchased from Sigma-Aldrich (Burlington, USA).

4.3.2 Preparation of the formulation

The prepared formulation is an oleogel with the two active pharmaceutical ingredients BDP and CA. The APIs were used in concentration of 0.64 mg BDP per gram oleogel and the CA in 0.05 mg per gram oleogel. The oleogel test formulation is a blend of castor oil 46.5 % medium chain triglycerides 46.5 % and the film-former OleoCraft™ MP-30 7 %, which were all molten up together at 100 °C in an oil bath. The mixture was then cooled to 65 °C. The APIs were placed in a melamin bowl and rubbed on with about 20 % of the oleogel. The remaining oleogel was added and stirred to combine. The mixture was filled warm so that the gel structure could form in the final container.[6]

4.3.3 Preparation of the porcine ear skin

Porcine ears were purchased from the Griesshaber butchery (Moessingen, Germany), and prepared following a literature procedure.[30] Briefly, the ears were bathed in isotonic saline and gently blotted using gauze balls size 6 (Fuhrmann GmbH, Much, Germany) to remove excess liquid. The skin was then separated from the porcine´s auricle and cleaned by using isotonic saline, then cut with a scalpel and stretched onto aluminum foil wrapped Styrofoam blocks and fixed with pins. The hair was trimmed to 0.5 cm (QC5115/15 Philips Electronics, NL-Eindhoven, Netherlands). Subsequently, 1.0 mm of the skin from lower tissue was removed using a Dermatom (Dermatom GA 630 Acculan 3 TI Aesculap AG & Co. KG, Tuttlingen, Germany). Finally, using a circular iron punch (07130250, Matador GmbH & Co. KG, Remscheid, Germany) 25 mm diameter skin samples were created, then wrapped in aluminum foil, and stored in a -28 °C freezer for maximum 6 months. On the day of the experiment the needed number of samples were thawed at room temperature.[30]

4.3.4 Fabrication of the microneedle patches

All MAPs were designed using Onshape. The three different needle shapes, conical, square pyramidal and obelisk were created with needle lengths of 400 µm, 600 µm, 800 µm, or 1000 µm. Tip angle was held constant at 14.5° for the conical and square pyramidal geometries, and 35° for the obelisk geometry. Needles were arrayed on a 14 mm diameter patch backing, with needle spacings held constant at 0.74 mm between needle tips, resulting in 209 needles per patch. MAPs were manufactured on a prototype S1 CLIP printer from Carbon, using a resin composed of PEG350DMA, 2.5wt% TPO

(photoinitiator), and 0.1wt% BLS (light absorber). Patches were printed using a slice thickness of 1 µm, speed of 12 mm/hour, light intensity of 4 mW/cm2 (λ = 385 nm). Following our previously reported procedures, the 3D printed microneedle patches were washed with isopropanol, air dried, and post cured for 5 min (Phoseon FireJet FJ800 ultraviolet light emitting diode, of 365 nm 60 W (LED) lamp.[25]

4.3.5 Strength testing of needles

To test how resistant the needles are to fracture, a texture analyzer (DO-FB0.5TS; Zwick GmbH &Co. KG, D Ulm) was equipped with two planar experimental dies with a diameter of 3.5 cm. The microneedle patch was laid down central on the lower die with the needles facing the upper die. The upper die moved continuously down to the lower die and stopped once the distance between the dies was 20 µm. The force curve and the distance of the dies was plotted against time. When the needles started to break, the force dropped for a short time. This peak represents the maximum force that the needles can withstand prior to fracture (n=3).[31]

4.3.6 Examination of skin puncture

Following microneedle application, skin was marked with tissue marking dye following literature procedure.[26, 32] Briefly, 500 µl of tissue marking dye (TMD, DCI´s #07228-2 Blue, Cancer Diagnostics, INC, Durham, NC, US) was mixed with 500 µl isopropanol in a 2 mL Eppendorf tube using a Vortex for 30 seconds. Following MAP application in ex-vivo porcine skin, the TMD solution was applied on the skin surface using a Q-tip, and allowed to air dry for 5 minutes. After drying, dye was removed from the skin surface using water-soaked Q-tips. The marked skin surface was then investigated using light microscopy (STEMI 350; Carl Zeiss AG, Oberkochen, Germany). Punctures sites were further analyzed to determine depth of puncture for the square pyramidal and conical needle designs (n=3).

4.3.7 Parafilm M® puncture

Parafilm M® was used as skin mimic.[33] To create a 1 mm thick testing film a sheet of Parafilm M® with a thickness of about 127 µm was folded eight times. The MAPs were pressed into the Parafilm M® layers with human thumb force. The layers were taken apart and examined for needle puncture using the light-microscope (n=3).

4.3.8 TEWL measurement

An auricular porcine skin sample with a diameter of 2.5 cm was placed into a Franz diffusion cell (Gauer Glas, Püttlingen. Germany) that was filled with phosphate-buffered saline (PBS) and incubated for 30 min in a water bath at 32 °C while stirring the buffer with 500 rpm. After equilibration time the Franz diffusion cell was placed outside the water bath at room temperature (22 °C and 25 % relative humidity) and 2 mL of the buffer was removed. The upper part of the Franz diffusion cell was removed, the skin blotted with a Kimtech tissue (Kimberly-Clark Global Sales, Inc., Roswell, USA), and the upper part of the diffusion cell replaced with the probe In-vitro-Tewameter® VT 310 connected to Multi Probe Adapter MPA 6 (Courage & Khazaka electronic GmbH, D-Colone, Germany) to measure trans epidermal water loss (TEWL). The measurement time was set to 90 s for each run.[34] Approximately ten measurements were performed before the TEWL readings remained stable (TEWL value ± 1.00 g/m x h) for three consecutive measurements. The skin sample was then removed from the Franz diffusion cell and treated with the microneedle patch for 5 s using force of thumb. The skin sample was then placed back on the Franz diffusion cell and the TEWL measured again until stable measurements were collected (TEWL value ± 1.00 g/m x h) for three consecutive measurements. This was repeated three times (three skin samples) for each MAP design.

4.3.9 Evaluation of the needles following application in ex-vivo porcine skin

MAPs were evaluated following application in ex-vivo porcine skin. Thumb force was simulated using a test stand with a 500N Digital Force Gauge (Mxmoonfree, HPB Test Stand+HP-500N, Seattle, United States). MAPs were applied to excised porcine skin using a constant force of 130 N for 30 seconds. MAPs were then removed, and the needles examined for damage using light microscopy (STEMI 350; Carl Zeiss AG, Oberkochen, Germany).

4.3.10 Ex-vivo skin penetration and permeation

Franz diffusion cells were used to evaluate skin delivery of BDP. A skin sample with a diameter of 25 mm was used for the experiments. The areas of both parts of the Franz diffusion cell that are in contact with the skin sample were covered with grease and a sample of the skin was applied. The chamber was filled with 12 mL PBS buffer (pH 7.4) and pre-incubated for 30 min at 32 °C to equilibrate. The Franz diffusion cell was placed within a heated water bath (Lauda type Alpha, Lauda Dr. R. Wobser GmbH & Co. KG, Lauda-Königshofen, Germany), on top of a magnetic stir plate (Variomag Poly 15,

Thermo- Scientific, Thermo Electron LED GmbH, Langenselbold, Germany). The acceptor-medium was stirred with a speed of 500 rpm. Following equilibration, 50 mg with 0.64 mg/g BDP of the test formulation were precisely applied on the skin using a plexiglass stamp, followed by microneedle patch application (force of thumb) for 30 seconds. The patch remained in the skin for the incubation time. To protect the setup from losing water, the top of the cell was covered with a piece of Parafilm M® and the sampling arm with a 1.5 mL Eppendorf tube.

After the incubation time of 8 hours the skin was removed, and the remaining formulation was wiped from the skin using swabs. This remaining drug was extracted from the swabs using a solution of methanol and water (1:1) in an ultrasound bath for 30 min. A skin sample of 1.5 cm was collected to obtain the treated portion of the skin, and frozen in liquid nitrogen. The drug permeating into the remaining skin ring was extracted in 2 mL of a 1:1 mixture of methanol and water in an ultrasound bath for 30 min.

Following a literature procedure, a cryo-microtome was used to slice and divide the different skin layers in the 1.5 cm skin sample.[6, 35] A total of 14 sections with a thickness of 16 µm were sliced for the stratum corneum, followed by 10 sections with a thickness of 20 µm for the epidermis, and 10 sections with a thickness of 80 µm for the dermis.[36] Drug from each section was extracted by incubating the tissue in 1.5 mL of a 1:1 mixture of methanol and water, on a shaker (Vibrax VXR basic, IKA GmbH & CO.KG, Staufen, Germany) (500upm) for 30 min at room temperature.

Drug from the acceptor-medium, representing drug permeation through the skin, was extracted in 15 mL of dichloromethane. After evaporation the residue was dissolved in 1 mL of a 1:1 mixture of methanol and water. The content of the API was analyzed using liquid chromatography in combination with mass spectroscopy (Varian 320-MS, Agilent Technologies, Santa Clara, USA). The samples were chromatographically separated with a reverse phase C8 column (EC 100/2 NUCLEODUR C8, Gravity 3 µm, Macherey- Nagel GmbH & Co. KG, Dueren, Germany) at a maintained temperature of 40 °C. The flow rate of the mobile phase was set to 300 µL/min with the flow agents water with ammonium formiate used in a concentration of 1 nM (A) and acetonitrile containing 1 nM formic acid (B). The separation started with a concentration of 30 % B and increased to a concentration of 70 % over 2.00 min. The concentration stayed constant over 5.00 min, then B was decreased back to the inital 30 % over 1.00 min. The total chromatographic run time was 8.00 min. The retention time of BDP was 4.94 min and it was detected with a triple-quadrupole after electrospray ionization. A capillary voltage of 30000 V and a

dwell time of 0.2 s were set and BDP was detected with an ESI positive mass of 411.3 m/z. The LOD for BDP was 0.316 ng/ml and the LOQ were 1.76 ng/ml with an r^2 of 0.9996.

4.3.11 Statistics

The experiments were conducted three or six times, as denoted in the respective methods descriptions. Statistical differences were determined by applying Analysis of Variance (ANOVA) followed by Student-Newman-Keuls test in Excel. In both tests $\alpha=0.05$ was as threshold of significance. For all analyses, ns indicates not significant, * indicates $p< 0.02$, ** indicates $p< 0.002$, *** indicates $p< 0.0002$, and **** indicates $p< 0.0001$.

4.4 Results and Discussion

4.4.1 Design and characterization of the microneedle patches

Herein, using CLIP 3D printing, we manufactured solid polymeric MAPs, designed to cover psoriasis plaques, and facilitate drug delivery of topically applied medications into the skin. We designed the MAPs with a thin, flexible backing so that they can conform to the area of the body onto which it they are applied. We evaluated MAPs consisting of three different needle geometries and four different needle lengths, with the goal of identifying a needle geometry and a needle length that affords robust delivery of therapeutics into the skin, without needle fracture, and with minimal skin damage, as psoriatic skin has a compromised skin barrier, making it more prone to infection.[37, 38] Patches were designed with either square pyramidal (P) (Figure 2A) , obelisk (O) (Figure 2B), or conical (C) (Figure 2C) needles arrayed across a 1.4 cm diameter patch (20 μm thick) (Figure 2C), resulting in each patch containing 209 needles on a thin and flexible backing (Figure 2D and 2E).[39] MAPs were designed to have needle lengths of 400 μm, 600 μm, 800 μm and 1000 μm. Based on initial print evaluations, CAD files were then scaled in the z-direction in an effort to manufacture needles of the appropriate dimensions (Table S1). Following manufacturing, all needle designs were evaluated using light microscopy (STEMI 350; Carl Zeiss AG, Oberkochen, Germany) to determine print fidelity, through measuring needle height, base width, and needles spacing, across 6 prints to establish reproducibility (Figure 3, Table S1). No significant difference regarding needle height, base width, and spacing was observed between print replicates, indicated high levels of print to print consistency.

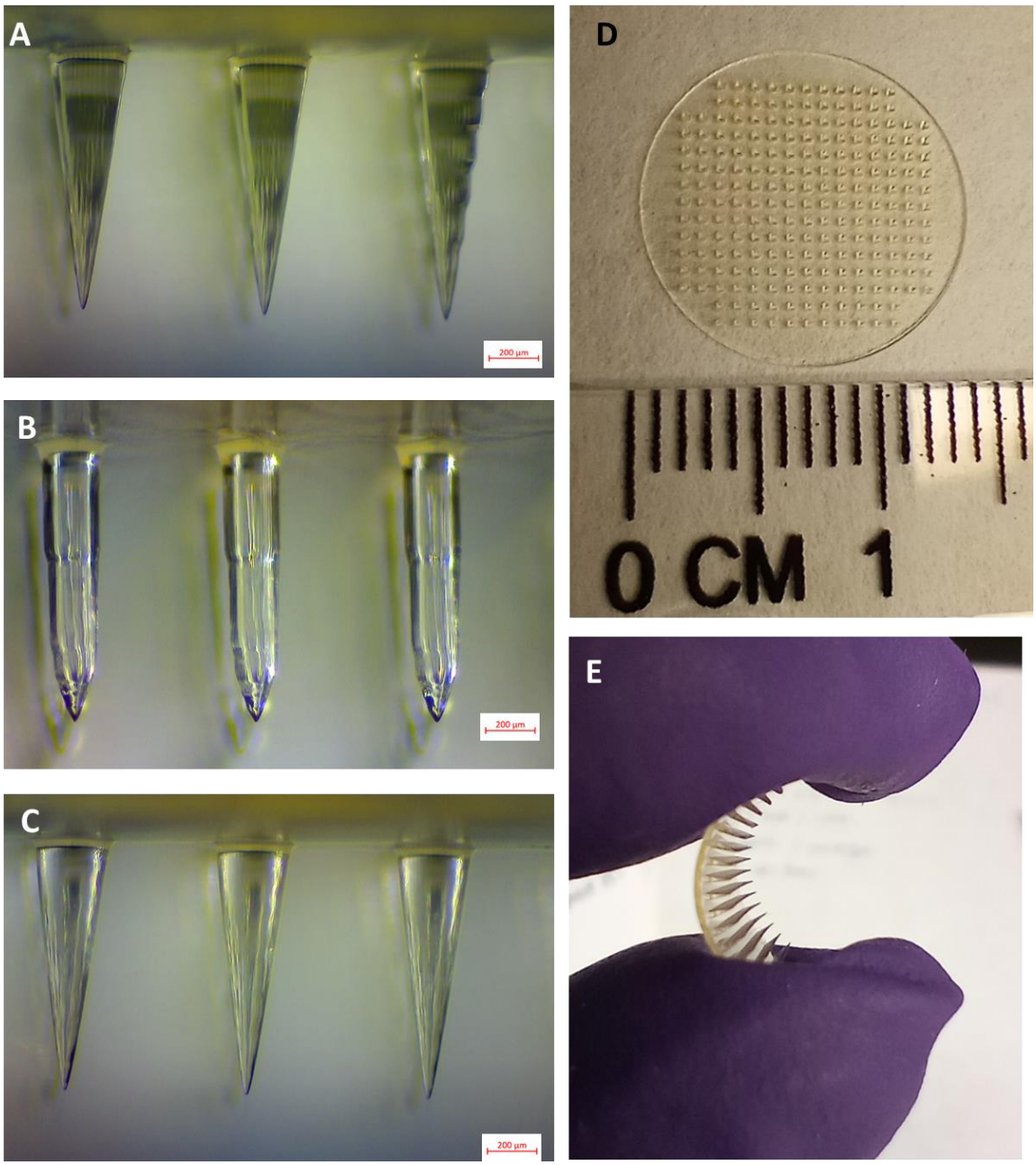

Figure 2: 3D printed Microneedle Array Patch. Representative light microscopy images of 1000 µm length needles of square pyramidal (A), obelisk (B), and conical (C), with scale bars representing 200 µm. Photographs of MAP flat (D) and compressed between two fingers (E) to show the flexibility of the base.

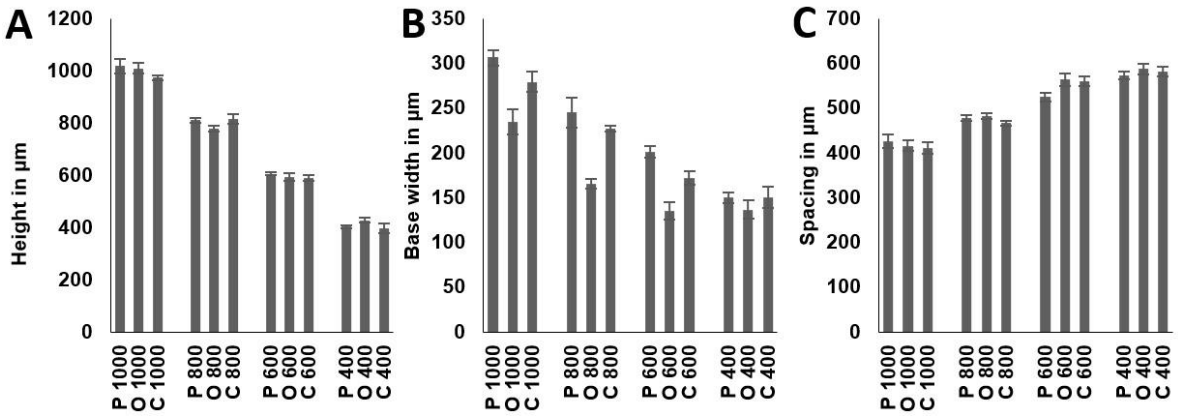

Figure 3: Microneedle Characterization. Microneedle patches of varied geometries and needle lengths were 3D printed and characterized using light microscopy to determine needle heights (A), base widths (B) and the spacing between needle bases (C). Results display the average of n = 6 with error bars representing the standard deviation.

4.4.2 Characterization of the needle strength

A texture analyzer was used to test the strength of the microneedles (method 4.3.5). As the upper die of the texture analyzer moves continuously downwards, the force acting on the needles increases until the needles break under the effect of the force. At this point, the measured force on the upper punch drops until it rises again after the needles have been crushed. Representative force curves generated from testing the three geometries is shown in Figure 4 A-C. Needle breakage was only observed with obelisk shaped needle patches, both the square pyramidal and conical shaped needles deformed or bent during these experiments and did not break or fracture, leading to inconclusive force to fracture information for these designs, using this method.

Surprisingly we did not observe a decrease in fracture force as a function of increasing needle length. Instead, we observed a trend of decreasing force to fracture as needle length decreased. We believe that this is due to the increased in base width for the longer needles. The base width decreases with needle length ranging from 230 µm for the 1000 µm tall needles down to 150 µm for both the 600 µm and 400 µm tall needles (Fig. 4B), corresponding to decreasing fracture forces with decreasing needle heights, shown in Figure 4D.

Based upon literature reports, the force that a person can exert with their thumb ranges between 100 - 130 N.[40] Based upon our experiments using the texture analyzer (Fig. 4D), only the 800 µm and 1000 µm long needles can withstand this force. However, this test

evaluated the needles being crushed into a firm surface, and thus we later evaluated the needles following skin application with a force of 130 N.

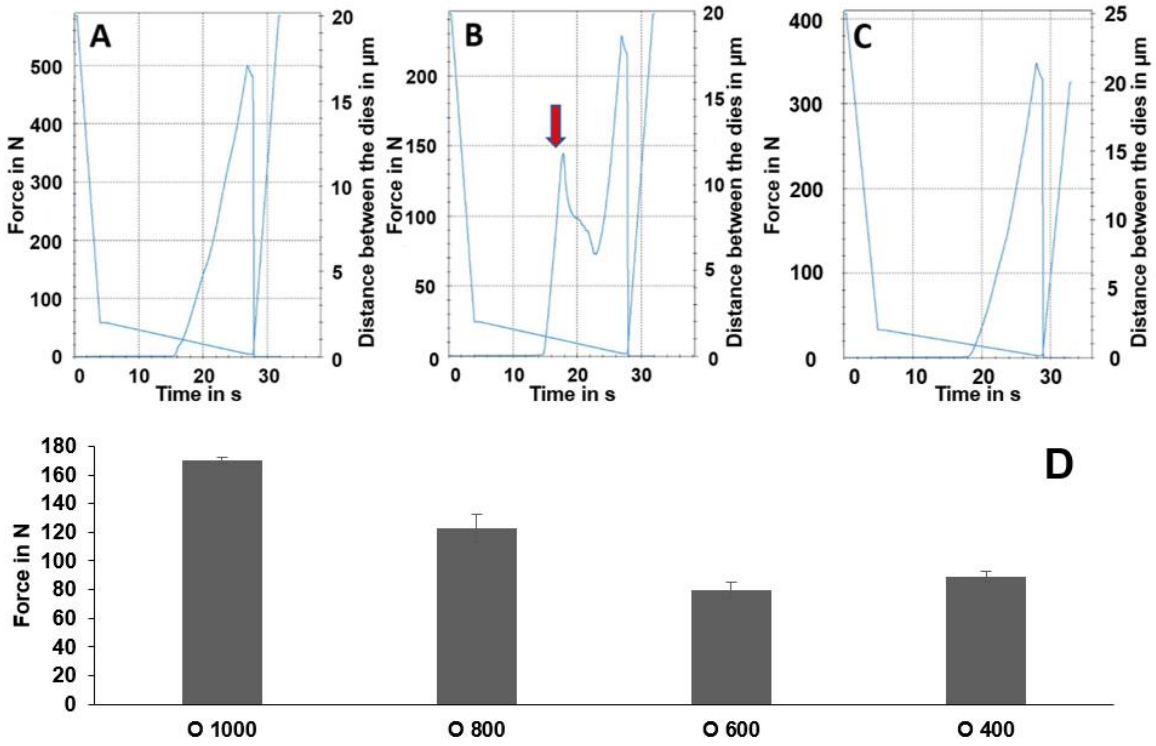

Figure 4. Evaluation of needle fracture force. Representative force curves for 1000 µm square pyramidal (A), obelisk (B), and conical (C) microneedle patches. The red arrow indicates the point of failure for the obelisk design. The breaking force of the needles can only be detected investigating the obelisk shaped MAPs (B) as indicated by the red arrow. Results of the strength testing of all obelisk microneedle patches with needles 400 µm (O 400), 600 µm (O 600), 800 µm (O 800) and 1000 µm (O 1000) in length (D), data represents force to fracture for each MAP design for n = 3; mean ± SD.

4.4.3 Skin puncture and penetration depth of the microneedle

Microneedle penetration depth was characterized following MAP application in ex-vivo porcine skin and Parafilm M®. Following MAP application in excised porcine skin, the puncture sites were marked with tissue marking dye fore ease in visualization using light microscopy and the diameter of the pores in the skin were measured (Figure 5). The pore sizes in the skin varied between 100 µm and 200 µm for all MAP designs. The measured base width of the individual microneedle designs is between 135 µm and 306 µm. As expected, we observed an increase in the average pore size trending with the increase in needle base width.

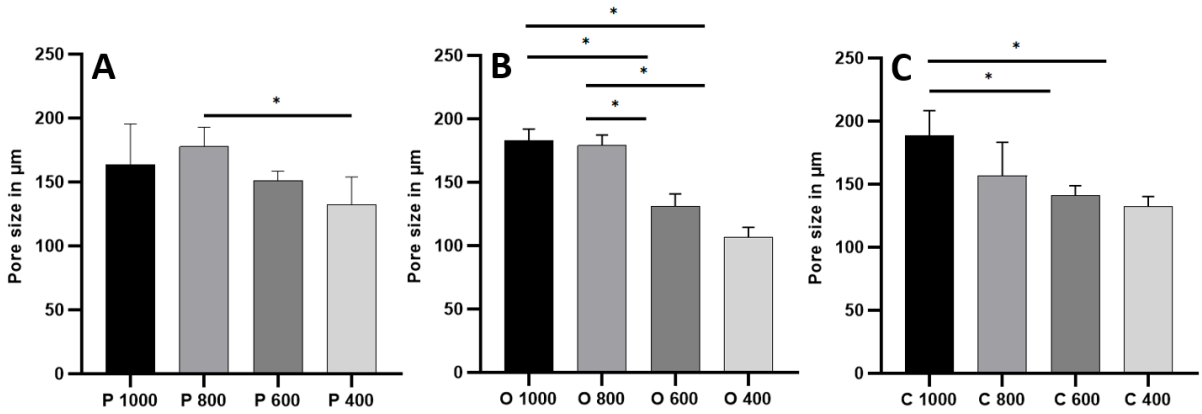

*Figure 5. Characterization of puncture sites following microneedle patch application. The pore size for each needle length for the square pyramidal (P) shaped MAPs (A), the obelisk (O) shaped MAPs (B) and the conical (C) shaped MAPs (C) is shown. n = 3; mean ± SD with * p< 0.02.*

Utilizing the known tip angle of the needle and measured pore size (from the skin puncture experiments) microneedle depth of penetration was calculated for both the square pyramidal and conical microneedles using the Pythagorean Theory (Figure S1), however this is not possible with the obelisk shaped microneedles, as the obelisk geometry exhibit constant needle width once the needle tip pierces the skin. As expected, we observed that the longer needles resulted in deeper penetration into the skin (Table 1). We further evaluated what percent of the needle length was embedded in the skin and found the proportion of the needle length penetrating the skin increased as the needle length decreased. We believe this may be a function of the "bed-of-nails" affect. While needle density and tip to tip spacing was held constant at 740 µm, as the needles get shorter the diameter of the base also decreases (Figure 2B), thus increasing the base to base distance between the needles (Figure 2C), and leading to an overall decrease in the area occupied by the needles as compared to the overall patch area. Previous studies have demonstrated that pressing microneedles into the highly elastic skin results in an initial skin indent prior to puncture,[41] we hypothesize that this increase in spacing between needle bases potentially allows the skin more space to deform, limiting compaction, and thus allowing for more of the needle length to penetrate the skin.

Table 1: Penetration depth of the different microneedle designs.

	P1000	C1000	P800	C800	P600	C600	P400	C400
Penetration depth in µm	546 ±106	659 ±70	590 ±50	564 ±95	456 ±23	486 ±58	358 ±59	351 ±22
Percent of needle length penetrating the skin in %	54.6 ±11	65.9 ±6.9	73.7 ±6.2	70.5 ±12	75.9 ±3.9	80 ±4.4	89.4 ±15	87.7 ±5.5

Previous literature reports have demonstrated that Parafilm M® can serve as a skin surrogate to determine depth of needle penetration.[42] Following the literature procedure, MAPs were inserted into 8 overlapping layers of Parafilm M® using thumb force.[43] Each Parafilm M® layer (approximately 127 µm thick) was then examined under the microscope and needle puncture sites counted (Figure S2, Figure 6). As expected, and in agreement with the skin puncture evaluation, longer needles were able to puncture deeper layers of Parafilm M®. None of the examined needle designs were capable of puncturing the sixth layer (762 µm) of Parafilm M®. All needle geometries and lengths were capable of puncturing the third layer of Parafilm M®, or approximately 381 µm deep. Furthermore, all geometries with needle lengths of 1000 µm were able to puncture the fifth layer of Parafilm M® (635 µm), whereas, only the 800 µm long square pyramidal needle geometry was able to puncture into the fifth layer of Parafilm M®. Both the conical and obelisk needles measuring 800 µm and 600 µm in length were only able to penetrate to the forth layer of Parafilm M® (508 µm). Whereas only the 400 µm long conical and obelisk needles were observed to puncture the fourth layer of Parafilm M® (381 µm).

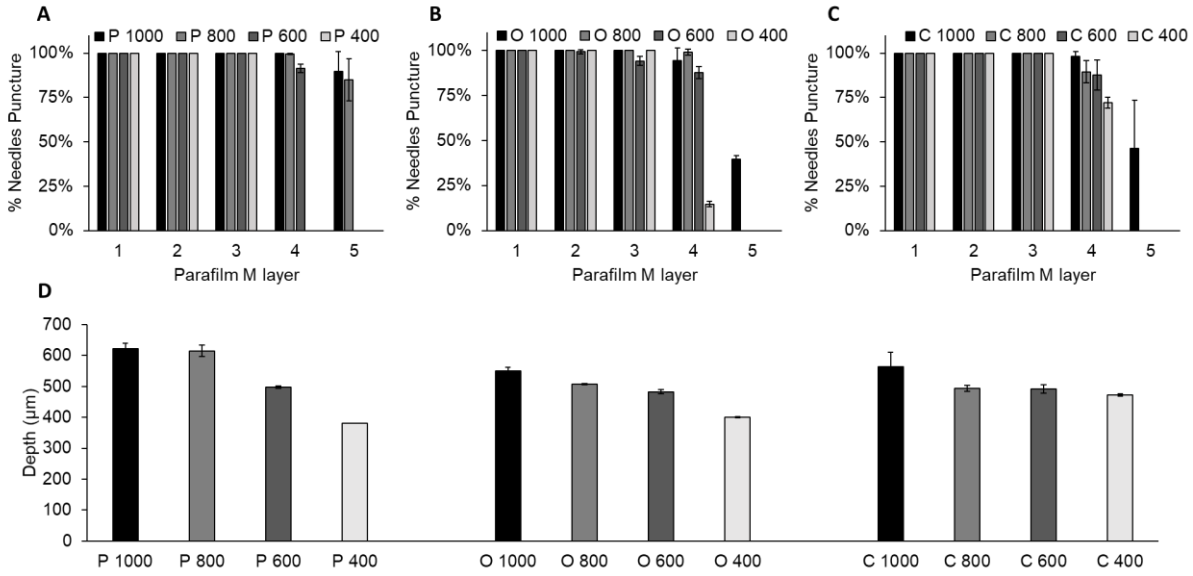

Figure 6: MAP puncture into Parafilm M®. MAPs were applied into 8 layers of parafilm using thumb pressure. Following MAP removal the number of puncture sites were

counted to determine the percentage of needles puncturing each layer for each MAP design (A) square pyramidal (P) MAPs, (B) obelisk (O) MAPs, and (C) the conical (C) MAPs (C). With the assumption that each parafilm layer is 127μm thick were then calculated the depth of penetration for each needle design (D). Results display the average of n = 3 MAP puncture tests with error bars representing standard deviation.

Comparing the results of needle puncture depth calculated from the ex-vivo porcine skin puncture experiments (Figure 4), with those obtained from the Parafilm M® puncture experiments (Figure 6) we found the data to be in agreement (Figure S3), with depth of puncture between the two methods deviating below 10 %, with no significant differences, which is in agreement with previous literature reports.[33]

4.4.4 TEWL measurements

Measuring trans epidermal water loss (TEWL) can give an indication of the skin barrier function, which mainly depends on the condition of the stratum corneum.[44] Since microneedles puncture the stratum corneum, we wished to evaluate water loss following treatment with MAPs to determine if we could mitigate water loss through microneedle design. A TEWL measurement between 2.3 and 44 g/m²/h depending on which part of the human body is measured is considered normal.[45,46] For ex-vivo porcine skin, we evaluated TEWL prior to MAP application, as well as after MAP application. TEWL measurements varied greatly ranging from 5 g/m2/h to 17 g/m2/h for untreated skin. For this reason, we quantified the change in TEWL for each skin sample following MAP application (Figure 7). As expected, we did see an increase in the change in TEWL readings ranging from 11 to 21 g/m²/h following MAP application. We observed limited changes in TEWL across needle lengths for the pyramidal and obelisk needle patches, whereas a trend of decreasing TEWL was observed for conical needles of decreasing lengths (Figure 7). We observed smaller changes in TEWL following skin application of the O 1000 μm MAP as compared to the P 1000 μm MAP, this could potentially be due to the difference in needle puncture depth, as we observed only 40 % of the O 1000 μm needles vs 80% of the P1000 μm needles were capable of puncturing to a depth of 635 μm. Interestingly, the increase in TEWL for skin treated with the C 600 μm MAP was significantly less than the P 600 μm patch, which is surprising as both these MAPs showed similar skin puncture depths (Figure7 and Table S2). Overall, it is important to note that all designs are well below TEWL readings of 44 g/m²/h, which is specified in the literature as value above which the skin is considered damaged.[45]

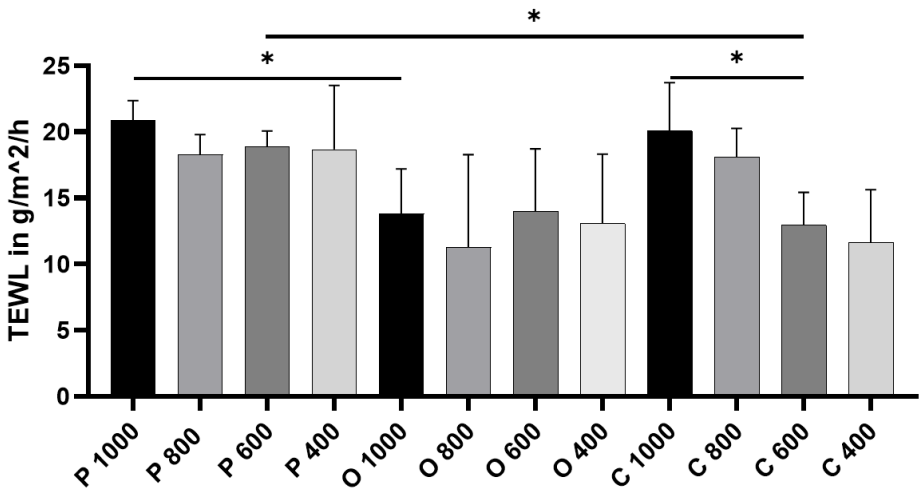

*Figure 7: Increase in TEWL after application of MAPs. For each skin punch, the TEWL value was measured before and after application of the MAPs, the bars represent the difference between the two values. n = 3; mean ± SD with * p< 0.02.*

4.4.5 Strength testing of the needles into the skin

Studies have shown that the maximum force a person can apply with their thumb is approximately 130 N.[40] To test whether the needles can withstand application in the skin, the microneedle patches were applied to a piece of porcine skin and pressed into the skin with a force of 130 N using a texture analyzer, as it is described in method 4.3.5. All needles of each patch have to resist the penetration into the skin without bending or breaking to ensure robust and repeatable skin puncture without potentially leaving fractured needles in the skin as this could result in inflammation. Following skin puncture tests, all needles of the entire MAP were viewed under a microscope, if one or more needles on the patch were bent or broken, the test was considered failed and displayed in Table 2 with a cross in red field. If all needles on the patch remained intact, the test was passed and displayed with a tick in a green field in Table 2.

Following skin application, we observed that the needle tips of the square pyramidal microneedles often broke, which could lead to some of the polyethylene glycol remaining in the skin. The reason for this could be that the surface of the needles was not completely smooth in some cases as can be seen in Figure 8A, resulting in breakage points on the needles, making it more likely for the needle tip to break off. Following skin application of the conical microneedles, we observed bent needles (Figure 8C), it is not possible to determine whether the needles were bent by the resistance before penetrating the stratum corneum or during penetration into the skin, but it can be stated, that they deform during skin application. Out of the three needle geometries explored,

only the obelisk microneedles, across all needle lengths remained intact following skin application (Figure 8B). Others in the literature have also explored obelisk needle designs, and observed that the obelisk needle geometry has higher mechanical strength than the square pyramidal-shaped microneedles.[19] Potential reasons for the improved mechanical strength of the obelisk design is that the needle base width decreases with a less pronounced angle towards the tip, giving the needles more volume, and the tip angle of the obelisk needle is also larger and is therefore supported by the very wide base.

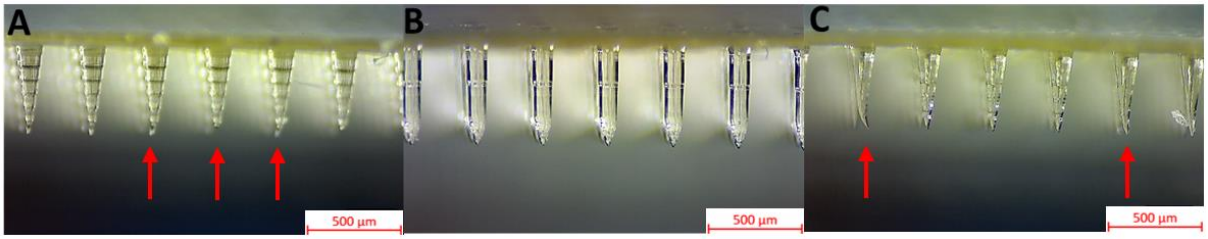

Figure 8: Examination of MAPs following skin application. MAPs were applied to the skin using maximum thumb force of 130 N. Following removal from the skin, the MAPs (A) square pyramidal, (B) obelisk, and (C) conical, were inspected using light microscopy. Scale bars represent 500 µm, and red arrows indicate broken or bent needles.

Table 2: Result of the stability testing while pressing the needles into the skin.

	Test 1	Test 2	Test 3
P 1000	✗	✗	✗
P 800	✗	✓	✓
P 600	✓	✗	✗
P 400	✗	✓	✓
O 1000	✓	✓	✓
O 800	✓	✓	✓
O 600	✓	✓	✓
O 400	✓	✓	✓
C 1000	✓	✓	✗
C 800	✓	✓	✗
C 600	✓	✗	✗
C 400	✗	✓	✗

4.4.6 Ex-vivo BDP delivery using Franz diffusion cell

Skin delivery experiments were conducted with MAPs designed with obelisk shaped microneedles with lengths ranging from 400 µm to 1000 µm. This design was down selected as it exhibited the best resistance to fracture and deformation following skin puncture. Ex-vivo porcine skin was treated topically with 50 mg of the film-forming

formulation that contained 32 µg of BDP, followed by MAP treatment. Skin was fixed within a Franz diffusion cell for eight hours, and the concentration of BDP remaining on the surface of the skin, delivered into the skin (stratum corneum, epidermis, and dermis) as well as in the permeate was evaluated as described in method 4.3.10, and the average recovery rate for BDP across all the samples collected was above 85% (Table S3). We observed significant improvements in dermal delivery with microneedles with lengths of 600 µm and higher (Figure 9), with no significant differences in the amount of BDP delivered to the stratum corneum, the epidermis, or permeate across all treatments. Further, we observed significant increases in BDP delivered into the skin for the 1000 µm needles as compared to the 400 µm, the 600 µm and the 800 µm needles. The delivered amount of BDP is also significantly increased using the 800 µm or the 600 µm MAP compared to the 400 µm MAP. The longer needles also have a thickened base-width proportional to their length. The resulting pore created in the skin not only reaches deeper into the skin, but also has a wider diameter at all points, which allows more of the drug to penetrate the skin via these channels. These results were therefore expected, as others in the field have also observed improvements in skin delivery as a function of increasing needle length (depth of skin penetration).[20]

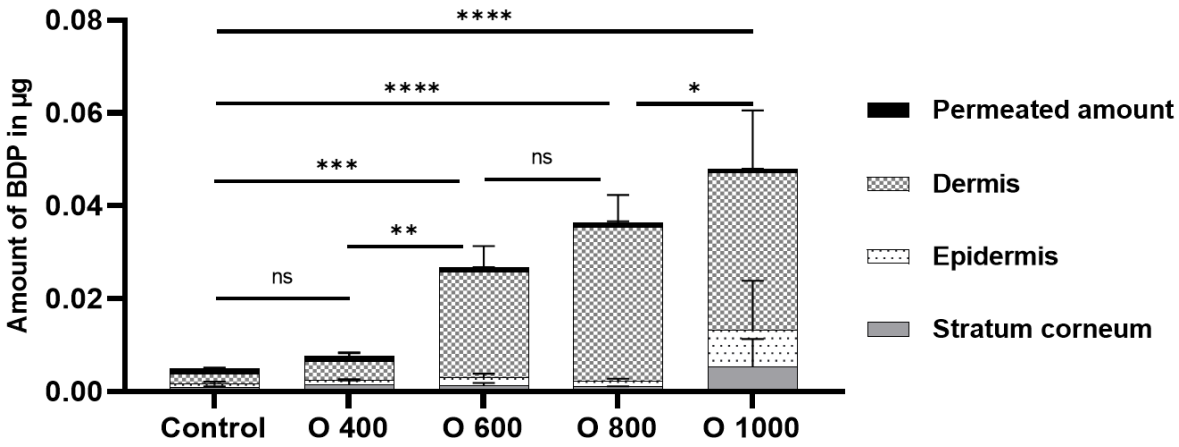

*Figure 9: Total penetrated amount of BDP into the different skin layers and throw the skin into the acceptor medium after 8 h incubation regarding different needle lengths of the obelisk shape compared to the control which is the formulation without any use of the microneedles; n = 3; mean ± SD, not significant (ns), * p< 0.02, ** p< 0.002, *** p< 0.0002, **** p< 0.0001 for total BDP delivered to the skin (dermis, epidermis, and stratum corneum).*

4.5 Conclusion

The aim of this study was to utilize CLIP 3D printing to manufacture and characterize MAPs of varied geometries and lengths in an effort to understand the role that needle geometry and length has on force to fracture, skin penetration, and dermal drug delivery. Through our characterization efforts, we found that while longer needles can penetrate deeper into the skin, the percent of the needle length penetrating the skin decreases as needle length increases. These observations are supported by findings from the Donnelly group.[12] Through optical coherence tomography, they illustrated that application of MAPs with shorter microneedles resulted in the base plate coming in closer contact with the skin than patches with longer needles, resulting in higher proportions of the shorter needles embedding in the skin as compared to the longer needles.[12] We believe this is due to the bed of nails effect[47, 48] and that future studies should focus on evaluating MAPs in which the needle design (geometry and length) is held constant and the distance between needle bases is increased. Increasing the distance between the needles allows more space for the displaced skin, reducing the impact of volume displacement caused by the needles to improve depth of penetration.[49] Furthermore, spacing can play a critical role in cargo distribution in the skin, and the total number of cells that the cargo reaches, further impacting therapeutic efficacy.[49]

We evaluated each needle geometry and length for stability following skin penetration using a maximum of 130N application force. From these studies we identified the obelisk needle design as the most robust, as this needle design across all needle lengths was able to remain intact following skin penetration, whereas we observed needle deformation for the conical and square pyramidal designs. While very few reports compared each of these designs within a single study, these observations are similar to those reported in the literature as Kim et al., reported on enhanced mechanical stability of obelisk microneedles as compared to square pyramidal microneedles.[19] They speculated that the enhanced mechanical stability was due to the larger cross-sectional area at a given height for the obelisk needles, generating less compressive stress per unit cross-sectional area for the obelisk needles, thus resulting in less deformation. Although the team did not investigate conical needles, these needles have similar cross-sectional area as the square pyramidal design.

Based upon the enhanced obelisk needle stability, we then utilized these obelisk MAPs to investigate the utility of MAPs in combination with our topical BDP-FFF. We found that MAPs of at least 600 μm were able to statistically increase the amount of BDP delivered

into the dermis, highlighting the benefit of combining FFFs and MAPs. Our findings are similar to those of Uppuluri et al., who reported that the amount of Zolmitriptan that penetrates the skin increases with microneedle length.[20]

While the 2016 pilot clinical trial resulted in enhanced efficacy when Daivobet® ointment was utilized in combination with 650 μm MAPs,[17] our data suggest that utilization of MAPs with longer needles could further improve treatment efficacy. These results form a basis for further studies focusing on evaluating the compatibility and efficacy of this combination using animal models of psoriasis.

CRediT authorship contribution statement

Larissa Carine Pünnel: writing- original draft, methodology, investigation, conceptualization. **Maria Palmtag:** methodology, investigation. **Jillian L Perry:** writing-review and editing, validation, methodology, investigation, supervision, conceptualization. **Dominique Jasmin Lunter:** writing –review and editing, conceptualization, resources.

Declaration of competing interests

The authors report no conflicts of interest in this work

Acknowledgments

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4.7 Supplementary Material

Table S1: Design parameter and measurements of the printed microneedle designs; n = 6; mean ± SD.

	CAD Design			Microscope			
	Height (µm)	Base width (µm)	Spacing (µm)	Height (µm)	Base width (µm)	Spacing (µm)	Tip-to-tip distance
C 400	700	180	560	397 ± 17	150 ± 12	583 ± 11	704 ± 6.7
C 600	900	228	512	591 ± 10	172 ± 7.9	560 ± 11	702 ± 6.2
C 800	1200	280	460	814 ± 19	227 ± 3.5	466 ± 5.3	711 ± 6.8
C 1000	1300	330	410	974 ± 8.6	279 ± 11	411 ± 13	712 ± 6.1
P 400	700	180	560	403 ± 6.1	150 ± 5.9	573 ± 9.3	695 ± 9.5
P 600	900	230	510	606 ± 5.3	202 ± 6.6	525 ± 9.7	672 ± 34
P 800	1100	280	460	810 ± 10	245 ± 17	477 ± 6.2	696 ± 22
P 1000	1300	330	410	1019 ± 28	306 ± 8.6	426 ± 14	687 ± 13
O 400	600	218	462	417 ± 8.9	137 ± 10	587 ± 13	699 ± 12
O 600	800	230	510	595 ± 14	135 ± 10	563 ± 14	702 ± 11
O 800	1000	280	460	779 ± 10	166 ± 5.8	482 ± 7.1	696 ± 6.8
O1000	1300	330	410	1009 ± 20	234 ± 14	416 ± 11	713 ± 7.0

The table shows how the dimensions were designed in the CAD model and how the dimensions turned out in the printed model that was investigated using the microscope. Each design was printed 6 times. The variations of the height, base width and spacing are not significantly different from each other. The tip to tip difference is not significantly different between the different designs.

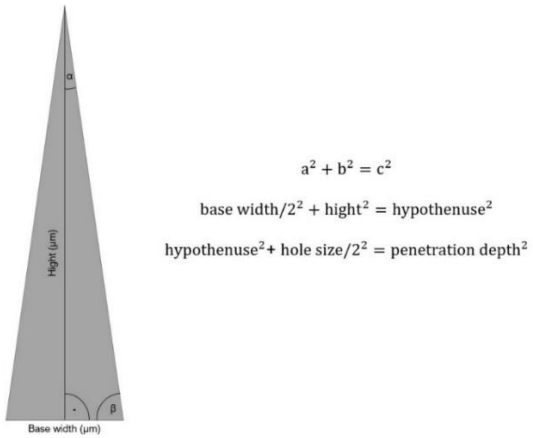

Figure S1: Calculation of the penetration depth

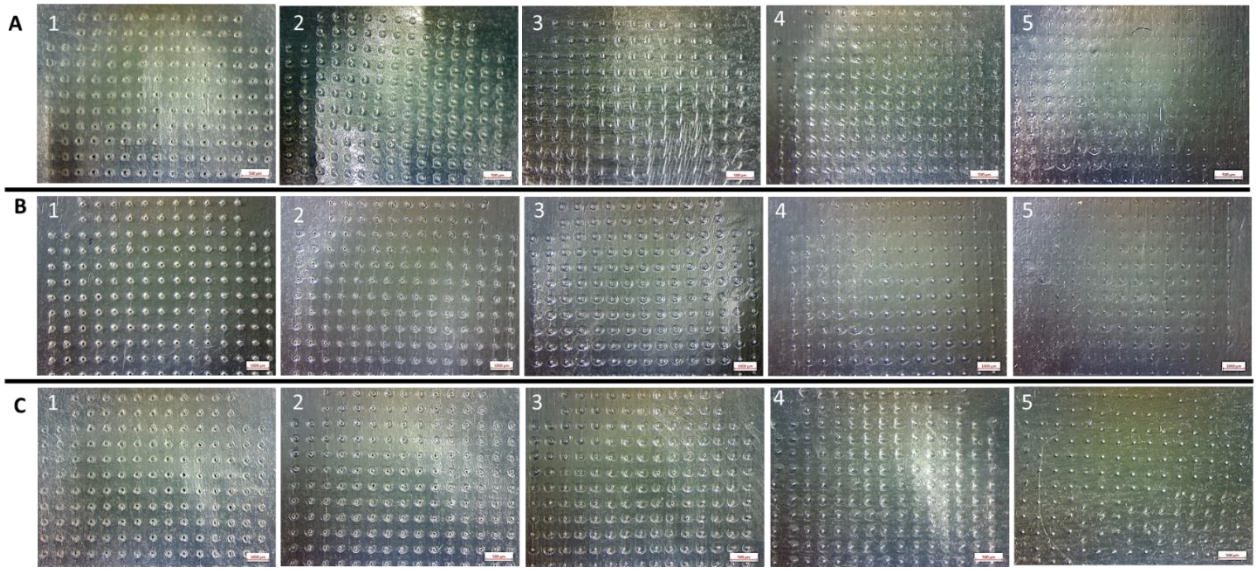

Figure S3. Parafilm M® Puncture Test. MAPs were applied to 8 layers of Parafilm M® with 130 N force. MAPs were removed, each Parafilm M® layer imaged using light microscopy, and puncture sites counted. Representative microscopy images for Parafilm M® layers following application with 1000 µm tall needles of either (A) square pyramidal, (B) obelisk, or (C) conical geometries. Scale bars represent 1000 µm.

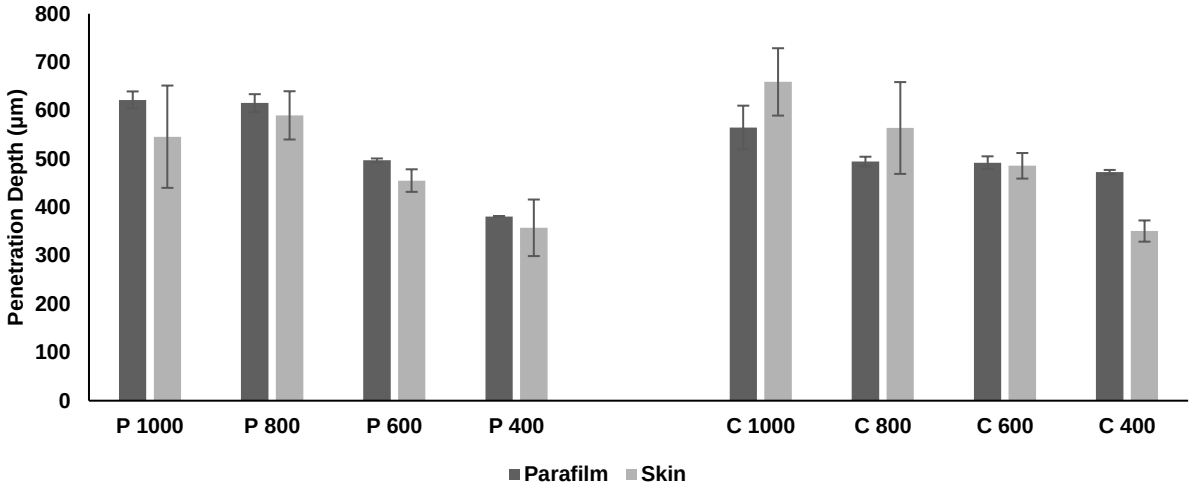

Figure S4: Comparison of penetration of ex-vivo pig skin to Parafilm M®. Calculated depths of penetration for needles applied in pig skin vs Parafilm M®. Penetration depth of the different microneedle designs comparing both testing methods, the pore size measurement with the calculated depth and the Parafilm M® testing.

Table S2: Measurement data of the trans epidermal water loss n = 3; mean ± SD.

	P1000 µM	O1000 µM	C1000 µM	P800 µM	O800 µM	C800 µM	P600 µM	O600 µM	C600 µM	P400 µM	O400 µM	C400 µM
Before Application	5.31 ±0.36	10.18 ±4.56	5.31 ±0.36	9.09 ±3.85	11.65 ±1.13	6.72 ±0.40	8.14 ±1.68	14.50 ±3.89	7.32 ±0.96	11.20 ±3.35	17.29 ±1.07	8.70 ±2.65
After Application	26.33 ±1.51	23.99 ±1.65	25.58 ±3.85	27.37 ±3.60	22.94 ±6.02	24.83 ±2.09	27.00 ±1.86	28.49 ±1.06	20.28 ±1.89	29.87 ±8.11	30.36 ±5.39	20.36 ±1.34
Difference	20.91 ±1.46	13.81 ±3.40	20.08 ±3.64	18.29 ±1.52	11.29 ±7.00	18.10 ±2.16	18.85 ±1.23	14.00 ±4.71	12.96 ±2.46	18.67 ±4.84	13.07 ±5.24	11.66 ±3.97

Table S3: Amount of BDP in different skin layers and the amount of BDP that was wiped of the skin surface after incubation in % together with the recovery rate of the BDP that was applied on each skin surface.

	Control 1	O 400 1	O 600 1	O 800 1	O 1000 1
Surface	99.99	99.98	99.96	99.92	99.91
Buffer	0.002777	0.001828	0.001739	0.002684	0.001569
Stratum corneum	0.001121	0.004387	0.003525	0.002891	0.02219
Epidermis	0.001120	0.002106	0.004107	0.004450	0.03647
Dermis	0.004000	0.01039	0.03368	0.06621	0.0334
Recovery rate	99.72	93.88	102.7	89.38	105.9
	Control 2	**O 400 2**	**O 600 2**	**O 800 2**	**O 1000 2**
Surface	99.99	99.99	99.95	99.91	99.90
Buffer	0.001706	0.003735	0.001716	0.001464	0.001552
Stratum corneum	0.002241	0.001262	0.003116	0.002025	0.002493
Epidermis	0.002838	0.001428	0.004312	0.001949	0.003352
Dermis	0.004299	0.003741	0.04537	0.08067	0.08771
Recovery rate	89.49	95.53	92.01	95.64	104.6
	Control 3	**O 400 3**	**O 600 3**	**O 800 3**	**O 1000 3**
Surface	99.99	99.98	99.93	99.92	99.89
Buffer	0.002752	0.001745	0.002189	0.002525	0.001625
Stratum corneum	0.002611	0.003881	0.001940	0.002416	0.006690
Epidermis	0.002004	0.002166	0.001446	0.002570	0.005066
Dermis	0.005977	0.01055	0.06185	0.07098	0.094230
Recovery rate	93.38	102.5	98.67	103.6	97.62

5. Stability study of the developed film-forming formulation for the treatment of psoriasis

5.1 Introduction

Every medicinal product must be tested for stability. The associated ICH Guideline "Q 1 A (R2): Stability testing of new drug substances and products - Step 5" provides information on the conduct of the study. In 1.3 it is written that "The purpose of stability testing is to provide evidence on how the quality of a drug substance or drug product varies with time under the influence of a variety of environmental factors such as temperature, humidity, and light, and to establish a re-test period for the drug substance or a shelf life for the drug product and recommended storage conditions."[1]

The storage conditions are different depending on the question if the formulation should be stored at room temperature, in the refrigerator or in a freezer. Semi solid dosage forms are usually stored at room temperature or in the refrigerator if it is needed to extend the shelf life. The storage conditions and recommended minimum storage time are shown in table 1.

Table 1: Storage conditions and recommended minimum storage time

Study	General use		Drug substance intended for storage in a refrigerator	
Long term	25°C ± 2°C / 60% RH ± 5% RH 30°C ± 2°C / 65% RH ± 5% RH	12 months	5°C ± 3°C	12 months
Intermediate	30°C ± 2°C / 65% RH ± 5% RH	6 months		
Accelerated	40°C ± 2°C / 75% RH ± 5% RH	6 months	25°C ± 2°C / 60% RH ± 5% RH	6 months

The testing frequency at accelerated storage conditions should include a minimum of 3 examination time points with 0, 3 and 6 months. During this time, the initial amount of the API should not change by more than 5% while an analytical error can be added. The formulation criteria like appearance, physical attributes, and functionality have to meet set requirements.

Due to the limited time period, examinations were carried out on day 0, day 7, after 1 month and after 3 months. The formulation was investigated with regard to changes in the rheological behavior. Both APIs BDP and CA were examined microscopically. The amount of BDP and CA was measured using HPLC.

5.2 Material and methods

5.2.1 Materials

BDP, castor oil and medium chain triglycerides were obtained from Caesar & Loretz, GmbH, Hilden, Germany and CA from Cayman, Ann Arbor, USA. OleoCraft™ MP-30 was kindly donated by Croda, Snaith, Great Britain. Parafilm was acquired from Brand GmbH & Co. KG, Wertheim, Germany, the microscope slides ground 45° and cover slips in 22x22mm from Epredia, Kalamazoo, MI 49008. Falcon Tubes in 15 mL and 50 mL were bought from Greiner, Kremsmünster, Austria. Sodium chloride, disodium hydrogen phosphate and potassium dihydrogen phosphate, ammoniumformiate and acetic acid were of European Pharmacopoiea grade. The used acetonitrile was acquired from Fischer scientific, Hampton, USA in HPLC Grade and ultrapure water (Elga Maxima) was produced in house. The package material tube was acquired from aponormn, WEPA Apothekenbedarf GmbH & Co KG, Hildscheid, Germany and the crock from Rink Pharmabedarf Gmbh, Donzdorf, Germany.

5.2.2 Preparation of the Formulations

The test formulation is an oleogel. Castor oil, medium chain triglycerides and the film-former OleoCraft™ MP-30 were molten at 100 °C in an oil bath. The mixture was cooled down to 65 °C. The APIs BDP and CA were weight into a second melamine bowl in the concentrations 0.64 mg/g BDP and 0.05 mg/g CA and about 20 % of this oleogel were used to form a pre-suspension. The rest of the mixture was then added and stirred before the temperature of the mixture fell below 55 °C. The mixture was filled into the final containers crock, tube or glass vial while warm so that the gel structure could form therein.

5.2.3 Storage conditions

After preparation the samples packed airless in the crock, the tube and the glass vial were stored for 1, 7, 28 days and for 3 months at 5 °C without a measured relative humidity (Rd 5000 Pure, Liebherr, Ochsenhausen, Germany), at 25°C with a humidity of 60 %, at 40 °C and 75 % (climate chamber Typ CB150, Fa. BINDER GmbH, DE-Tuttlingen) and in a climate chamber with changing conditions at -5°C 10h up to 40 °C over 2 h and 40 °C for 10 h and back to – 5 °C over 2 h. The entire cycle takes 24 h (Typ 3401, Rubarth Apparate GmbH, DE-Laatzen).

5.3.4 Rheological Measurements

Oscillation measurements were performed by a plate-to-plate geometry rheometer (Anton Paar MCR 501) with a 2.5 cm diameter plate with a gap size of 0.2 mm. The lower plate was tempered to 32 °C. The conditions are shown in Table 2.

Table 2: Conditions of the oscillation measurements.

Phase	**Condition**	**Duration (s)**
Pre-shear	Shear rate = 5 s−1	40
Rest	-	120
Amplitude sweep	Logarithmically from 0.01% to 100%; 1 Hz	600

To distribute the formulation evenly and prevent measurement errors, the formulation is pre-sheared for 40 seconds. The formulations were then allowed to rest for 120 s so that the structure could be rebuilt for the actual measurement. The amplitude sweep measurement was conducted with a deformation between 0.01 and 1000 %. The storage modulus (G') and the loss modulus (G'') were measured with a frequency of 1Hz every 6 seconds for 100 points. The storage module and the loss module were plotted against the amplitude within the linear viscoelastic range (LVR), the loss factor tan α (G''/G') and the yield point (G'=G'') were determined to describe the gel character of the formulations and to determine differences over storage time.

5.3.5 Polarization Microscopy

For investigation of the formulation the Axio Imager Z1 microscope (Carl Zeiss, Jena, Germany) with crossed polarizers with a ¼ λ-plate was used to investigate the formulations using a magnification of 20x (numeri-cal aperture 0.8, Plan-Apochromat, Carl Zeiss AG, D-Oberkochen, Germany). Those were applied in a thin layer to the microscope slide and covered evenly with a coverslip.

5.3.6 Determination of drug content

For the quantification of BDP and CA, HPLC (Shimadzu setup (CBM- 20 A, SIL- 20A, LC-20AT, DGU- 20A SR) with a UV/Vis detector (SPD- 20 A, Shimadzu, Kyōto, Japan)) was used. With a C18 reverse-phase column (EC 125/4 NUCLEOSIL 100-5 C18, Macherey-Nagel GmbH & Co. KG, Dueren, Germany), which was temperated at 35°C. An isocratic method was performed with acetonitrile and water 35:65 (V: V) and a flow rate of 1 ml/ min for 80 minutes. The injection volume was 10 µl. The BDP was detected after 72 min

at a wavelength of 230 nm. The coefficient of determination r^2 for the calibration was 0.9940. The calculated limit of detection (LOD) was 14.92 µg/ml and the calculated limit of quantification (LOQ) was 51.97 µg/ml. The CA was detected after 61.5 min at a wavelength of 230 nm. The coefficient of determination r^2 for the calibration was 0,9949. The calculated limit of detection (LOD) was 2.51 µg/ml and the calculated limit of quantification (LOQ) was 8.57 µg/ml. The amount of both APIs was tested using a one-sided t test (α=0.05) with a confidence limit for the mean curve that represents the acceptance criterion.[1]

5.4 Results and Discussion

5.4.1 Rheological characterization

The stored oleogels were measured by oscillation rheometry. Figure 1-3 show the results of the oscillation measurements for all stored samples. To determine whether the formulation changes rheological properties under storage conditions, the loss factor in the linear viscoelastic region, i.e. the difference between the dynamic and the loss module were compared with each other. Additionally, the yield point was determined as the crossover point of the dynamic modulus G' and the loss modulus G".

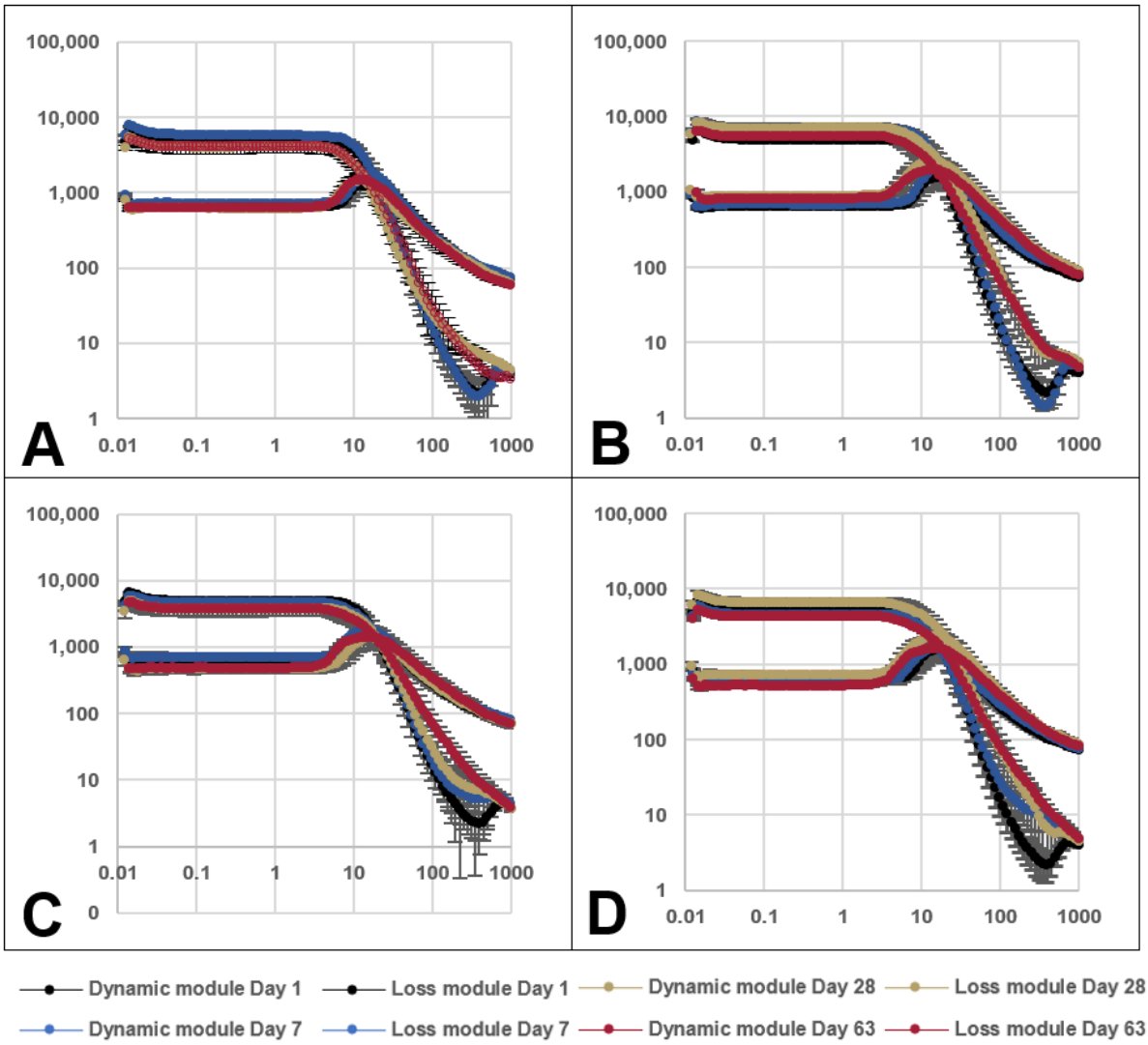

Fig. 1: Results of the rheological characterization of the film-forming formulations stored in a crock at 8 °C (A), stored at 25 °C, 60% RH (B), 40 °C 75% rh (C) and between -5 °C and 40 °C (D) for 3 months. (n=3, mean ± SD).

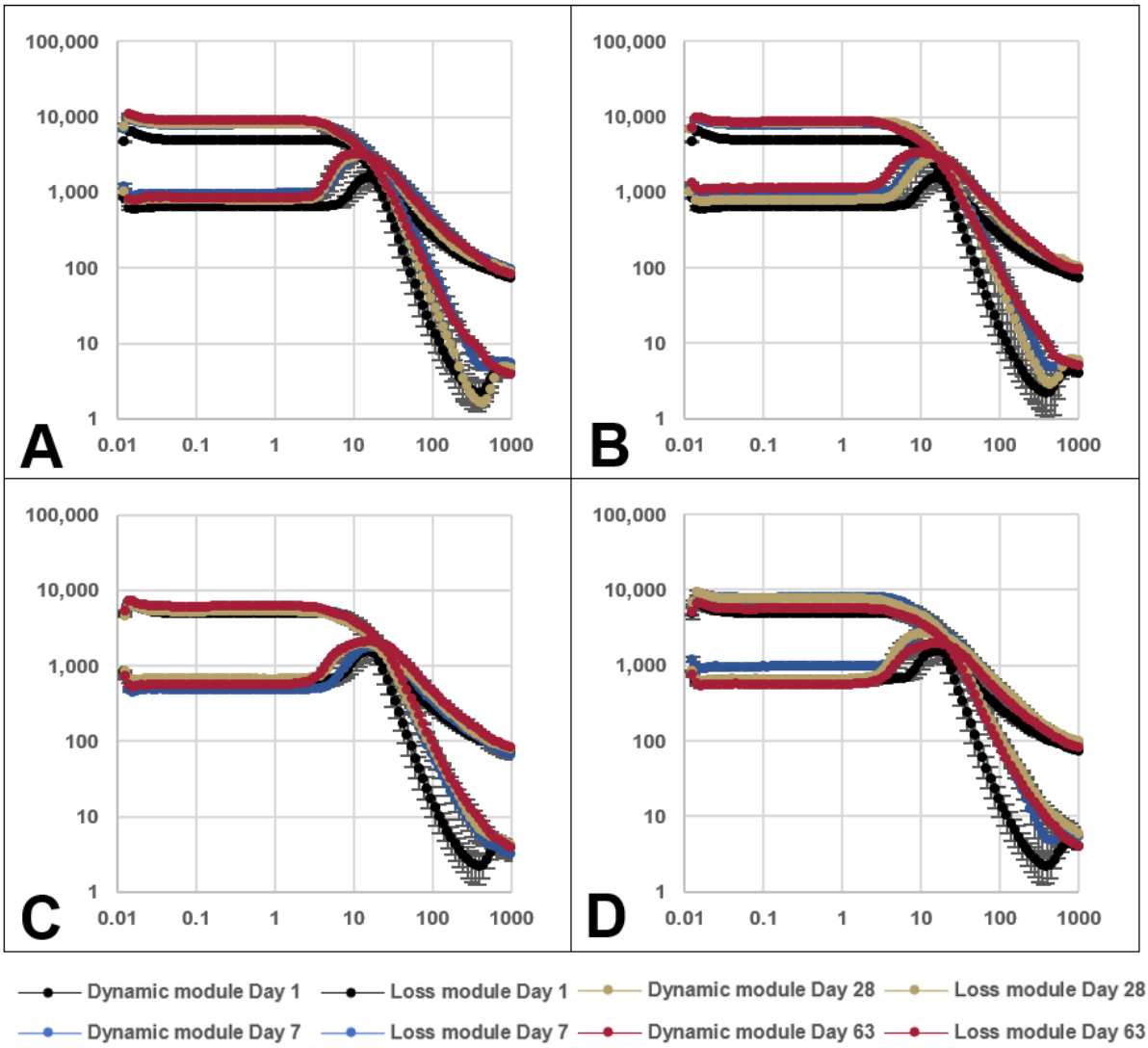

Fig. 2: Results of the rheological characterization of the film-forming formulations stored in a tube at 8 °C (A), stored at 25 °C, 60% RH (B), 40 °C 75% rh (C) and between -5 °C and 40 °C (D) for 3 months. (n=3, mean ± SD).

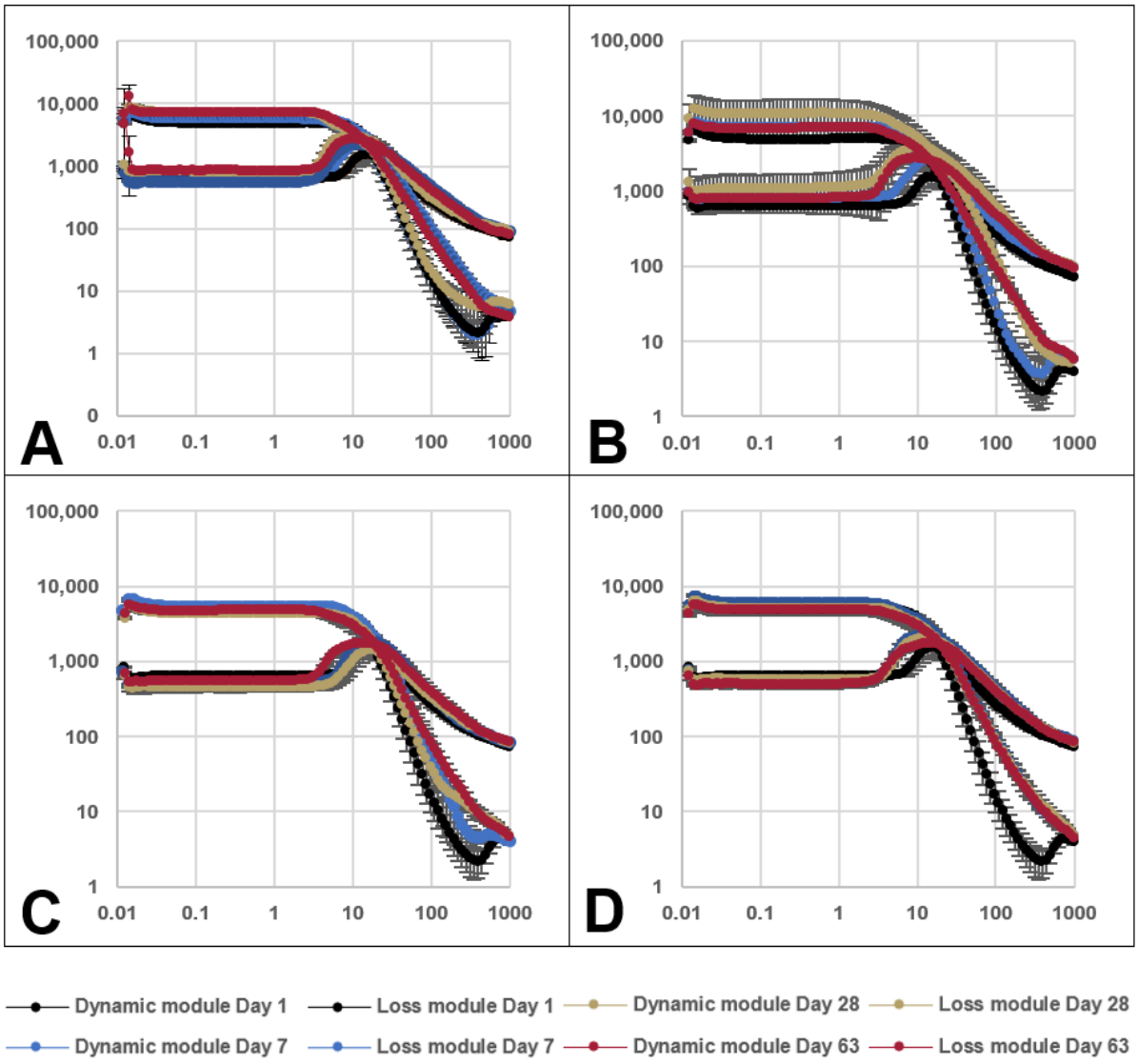

Fig. 3: Results of the rheological characterization of the film-forming formulations stored in a glass vial at 8 °C (A), stored at 25 °C, 60% RH (B), 40 °C 75% rh (C) and between -5 °C and 40 °C (D) for 3 months. (n=3, mean ± SD).

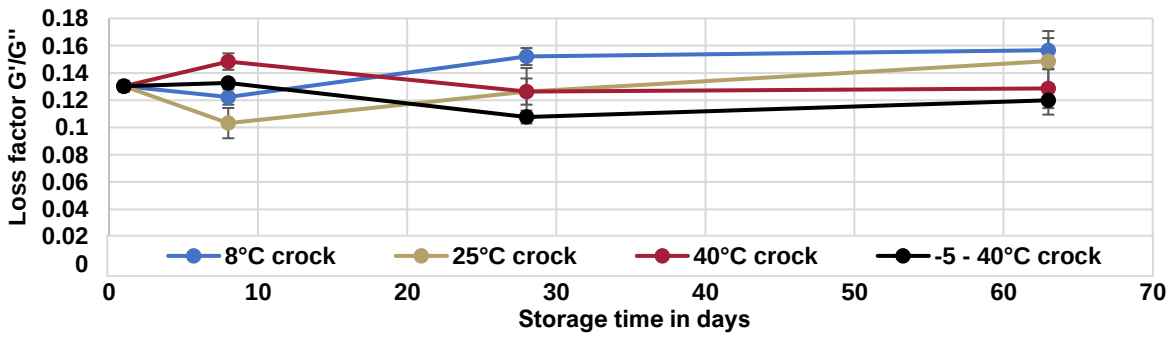

Fig. 4: Loss factor over time of the formulations stored in the tube at different temperatures. (n=3, mean ± SD).

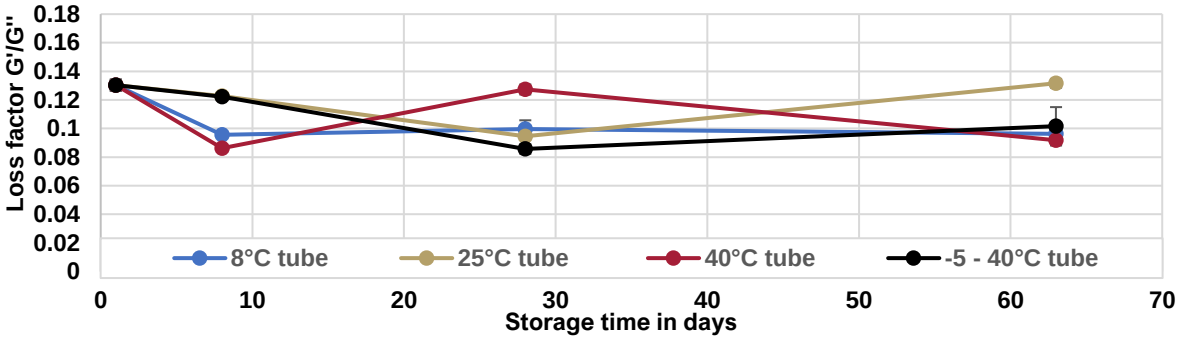

Fig. 5: Loss factor over time of the formulations stored in the tube at different temperatures. (n=3, mean ± SD).

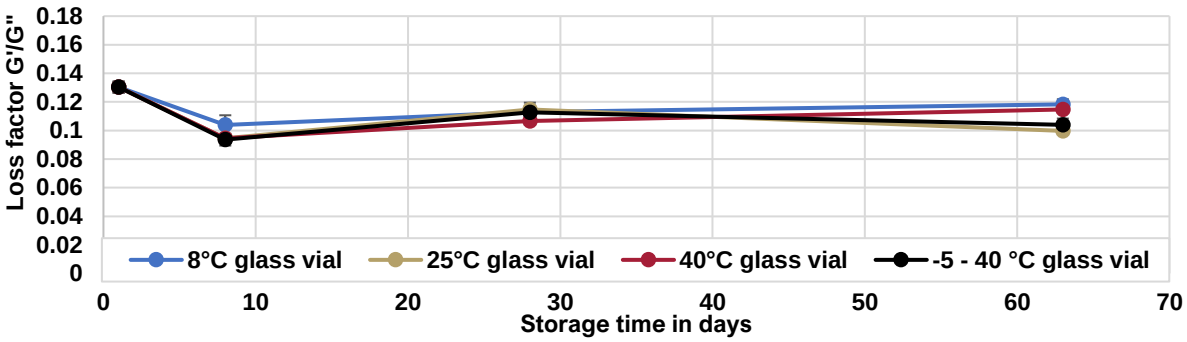

Fig. 6: Loss factor over time of the formulations stored in the glass vial at different temperatures. (n=3, mean ± SD).

Both, the yield point and the loss factor varied from sample to sample without a clear tendency for a loss factor change as a function of the storage temperature. A significant difference between day 1 and each storage time could not be determined. Therefore, it can be assumed that the rheological properties of the formulation do not change over the storage period of 3 months at 8°C, 25°C 60%RH, 40°C 75° RH and between -5°C and 40°C in the three tested packaging materials.

5.4.2 Microscopical characterization

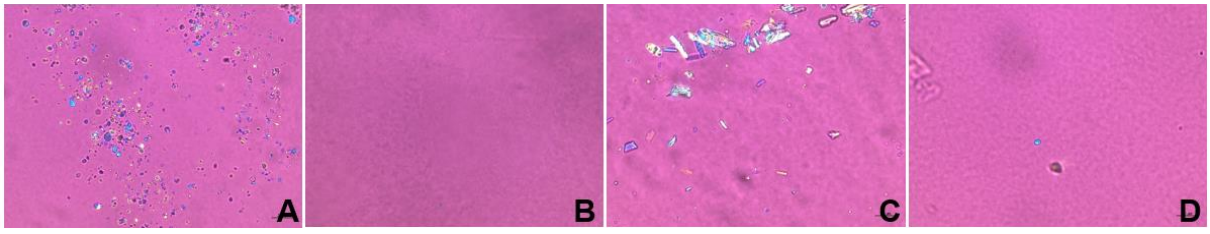

Fig. 7: Micronized betamethasone dipropionate in the film-forming formulation (A), reference of the film-forming formulation (B), micronized calicpotriole in the film-forming formulation (C) and an example of how the particles appear in the formulation (D).

Small BDP and CA particles could be detected in the formulation in all samples. No crystal growth could be detected over storage time.

5.4.3 Quantification of betamethasone dipropionate and calcipotiole after storage

The amount of BDP and CA was determined using HPLC. According to the ICH Q 1 A (R2) guideline, the active ingredient content must not decrease by more than 5 % over time. The amount of BDP and CA was detected on the day of production. Figures 8 and 9 show the amount of BDP and CA that were detected in all samples at the three storage times after 7, 28 and 63 days.

Regarding the amount of BDP in the stored formulations in the tube, no degradation below 95% could be detected. The amount of BDP in the formulation stored in the crock remained above 95 % when stored at 8°C, 25°C 60% RH and 40°C 75% RH. When stored in the climate chamber changing the temperature between the -5°C and 40°C the amount decreased over time below 95%, but since the amount was still in the acceptable range of the confidence interval it can be state that the formulation remained stable.

The content of BDP in the formulations stored in the glass vial and stored in the climate chamber was also below 95% after 7 days. Since the content increased above 95% after 28 days there is a possibility of measurement error. The confidence interval here is also still within the acceptable range, it can therefore be assumed that the BDP in the formulation was stable.

The amount of CA did not decrease under the mark of 95% on average in the stored formulation except of the formulation stored in the crock under temperature cyle testing. Also here, no amount of CA could be found outside the confidence interval, so it can be assumed that the amount of CA in the formulation was stable.

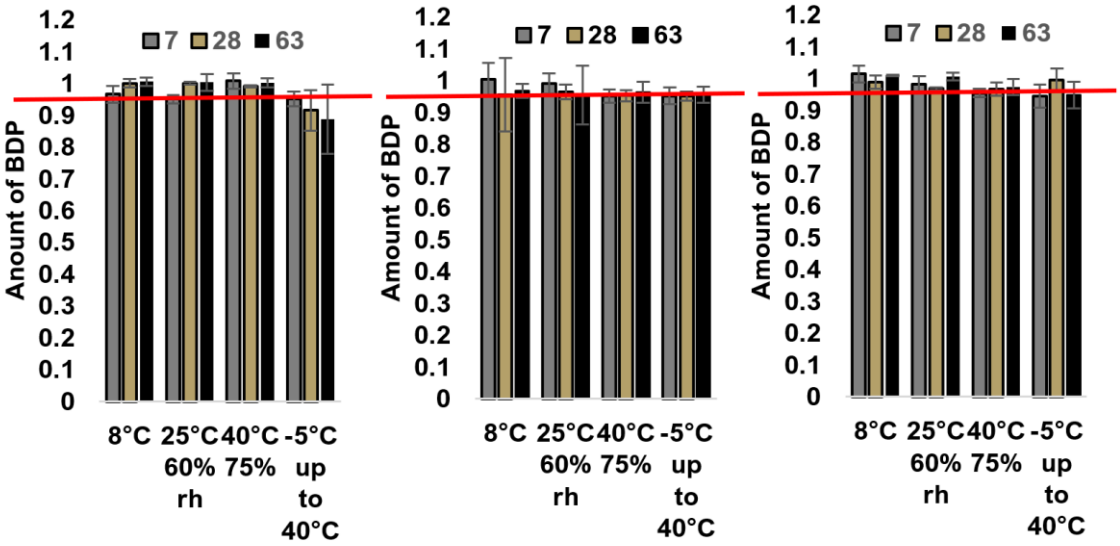

Fig. 8: Amount of BDP after 7, 28 and 63 days of storage in the crock, tube and glass vial. (n=3, mean ± SD)

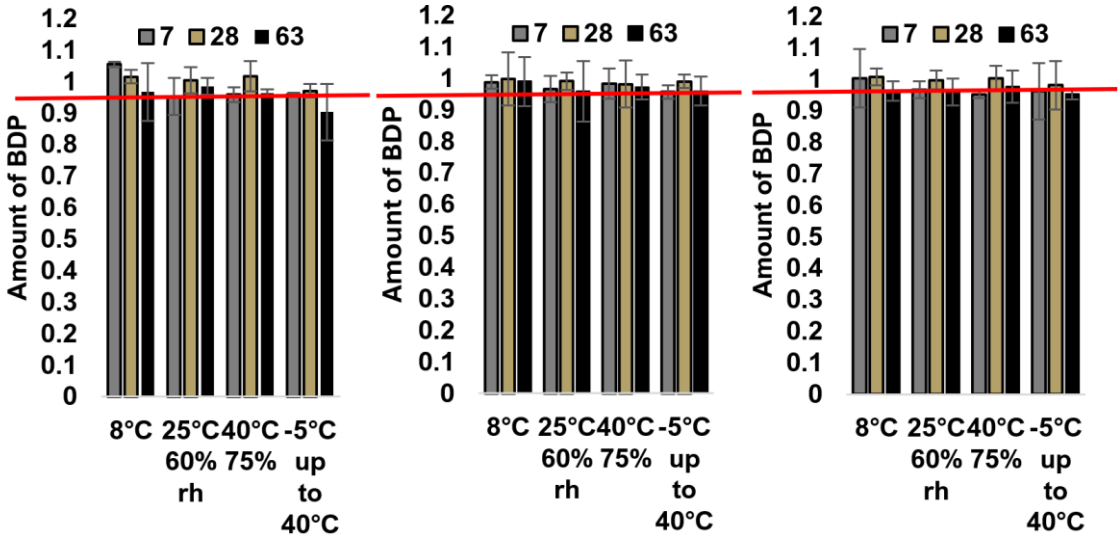

Fig. 9: Amount of CA after 7, 28 and 63 days of storage in the crock, tube and glass vial. (n=3, mean ± SD)

5.4 Conclusion

In conclusion, the stability study regarding the film-forming formulation with 0.64 mg/g BDP and 0.05 mg/g CA over a 3-month storage period demonstrated that both compounds remained stable under the conditions tested. No significant deviations in the amount of BDP and CA were observed, as the measured amounts consistently fell within the predefined confidence intervals. Additionally, the microscopic analysis revealed no notable changes in the physical appearance or structure or size of both, BDP and CA.

The rheological measurements confirmed that the formulation maintained its consistency.

These findings show that the combined formulation of BDP and CA is stable for at least 3 months. In order to make statements over a longer period of time and to verify the stability of the storage conditions used in this study, a stability study over a longer period of time would need to be carried out.

5.5 References

(1) European medicines agency: ICH: Q 1 A (R2): Stability testing of new drug substances and products – Scientific guideline, 2023 (ICH Q1A (R2) Stability testing of new drug substances and drug products - Scientific guideline | European Medicines Agency (EMA) (europa.eu))

6. General conclusion

The main objective of this work was to develop a semi-solid formulation for the treatment of psoriasis with a high content of lipophilic components that remains on the skin surface for an extended period of time due to an enhanced substantivity.

A formulation made of castor oil, medium-chain triglycerides, and the OleoCraft™ film former MP-30 was successfully developed, showing an improved substantivity compared to the in-market products Daivobet®, Enstilar®, Wynzora®, and Xamiol®.

Since the active ingredient combination of 0.64 mg/g BDP and 0.05 mg/g CA has established itself as the gold standard for topical treatment, this combination was also chosen to be used in the developed formulation. The two active ingredients should penetrate the skin out of the formulation in comparable quantities to the in-market products. Ex-vivo penetration studies using porcine skin demonstrated that the amount of BDP penetrating into and through the skin is comparable to that of the tested in-market products. However, the amounts of CA that penetrate the skin could not be detected in this study due to the fact that the CA skin penetration was beyond the detection limits of the study's analytical methods.

It could also be shown that the quantities of BDP penetrating the skin change when the test setup is combined with the one of the substantivity test. Following three hours of repetitive textile contact and an additional nine-hour incubation, BDP skin penetration was significantly reduced compared to conditions without textile contact across all tested formulations, but the developed formulation showed superior penetration relative to two of the in-market products. Minimizing product loss to clothing and other skin areas not only enhances the therapeutic outcome by prolonging skin contact but also reduces the risk of exposing others' healthy skin to drug and potentially resulting drug-related side effects.

Furthermore, the use of MAP was explored to further improve the therapeutic effectiveness of the topical treatment. Microneedles facilitate the penetration of the APIs by creating microchannels through the thick psoriasis plaques. In this study, obelisk-shaped microneedles, measuring 600 µm to 1000 µm in length were 3D-printed using CLIP and lead to an increased BDP skin penetration.